













NATIONAL INSTITUTES OF HEALTH

Report for the Fiscal Year

July 1, 1950 --- June 30, 1951

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FEDERAL SECURITY AGENCY  
Public Health Service



N A T I O N A L   I N S T I T U T E S   O F   H E A L T H

Unabridged report for the fiscal year

July 1, 1950 --- June 30, 1951

William H. Sebrell, Jr., Director  
Norman H. Topping, Associate Director  
David E. Price, Associate Director

	Page
NATIONAL CANCER INSTITUTE . . . . .	3
NATIONAL HEART INSTITUTE . . . . .	47
NATIONAL INSTITUTE OF DENTAL RESEARCH . . . . .	103
NATIONAL MICROBIOLOGICAL INSTITUTE. . . . .	111
NATIONAL INSTITUTE OF MENTAL HEALTH . . . . .	149
NATIONAL INSTITUTE OF ARTHRITIS & METABOLIC DISEASES . .	177
NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES & BLINDNESS .	209
DIVISION OF RESEARCH GRANTS . . . . .	213
RESEARCH FACILITIES PLANNING BRANCH (Clinical Center) . .	225



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## INTRODUCTION

The development of major research tools within the past decade has often demanded a rapid expansion or shift in research programs to take advantage of those scientific findings which open up new areas of investigation on diseases not previously subject to deep exploration. The synthesis of ACTH and cortisone is a case in point; their discovery opened the way for exploration of arthritis and rheumatism as well as many other diseases and problems of the human body.

With the passage of Public Law 692 by Congress in August 1950, administrative flexibility in meeting public health needs through the Public Health Service's research programs at the National Institutes of Health has finally been realized. The Surgeon General of the Public Health Service is now authorized to establish, when necessary, one or more additional institutes, or to expand the functions of already existing institutes of the National Institutes of Health so that future adjustments to research progress affecting the public welfare may be made with maximum effectiveness and rapidity.

Present needs for supplementing and expanding the research programs of the Public Health Service were also met by Public Law 692. By this law two new institutes were officially established -- the National Institute of Neurological Diseases and Blindness and the National Institute of Arthritis and Metabolic Diseases, the latter assuming the functions of the old Experimental Biology and Medicine Institute, which was thereby abolished. The addition of these new institutes initiates a broader program of scientific investigation into such metabolic diseases as arthritis and rheumatism and into such neurological and sensory diseases as cerebral palsy, epilepsy, multiple sclerosis, and glaucoma and cataracts -- the major causes of crippling and disability in this country.

The further effectiveness of the Public Health Service's intramural research program was assured by another provision of Public Law 692. Authorization of 30 additional positions in the professional and scientific service at the highest salaries -- from \$10,000 to \$15,000 -- means that the Service will be able to recruit outstanding scientists outside the government, and will also be able to keep those men already in the Service who have been offered more lucrative positions elsewhere.

The structure of the Public Health Service's extramural program was also altered for greater flexibility and effectiveness by the same law. The membership of the Advisory Councils, which, subject to the Surgeon General's endorsement, approve medical research grants to non-Federal institutions and universities, was stabilized so as to include six leaders in public affairs

and education as well as six distinguished scientists. Many of the policies, therefore, which govern the distribution of grants funds -- almost \$18,000,000 in 1951, or approximately 1/4 of all non-Federal medical research -- are determined by well balanced groups with a broad knowledge and understanding of both scientific and public needs.

On October 1, 1950, Assistant Surgeon General R. E. Dyer retired after 34 years in the Public Health Service. During the last 8, he had served as Director of the National Institutes of Health and guided the expanding research program at Bethesda, Md., with wisdom and distinction. Dr. Dyer's successor is Dr. W. H. Sebrell, Jr., formerly Director of the Experimental Biology and Medicine Institute.



NATIONAL CANCER INSTITUTE

John R. Heller, Director

C O N T E N T S	Page
Introduction.....	3
Technical Services.....	3
Biometrics Section.....	3
Documentation Section.....	4
Cancer Reports Section.....	5
Cancer Research Branch.....	5
Biochemistry Section.....	6
Enzymes and Metabolism Unit.....	6
Clinical Biochemical Research Unit.....	7
Cytochemistry Unit.....	8
Metabolism and Growth Unit.....	9
Nutrition Unit.....	11
Biology Section.....	11
Biological Studies Unit.....	11
Cell Adaptation Unit.....	13
Cell Chemistry Unit.....	13
Developmental Physiology Unit.....	14
Gastric Cancer and Tumor Immunity Unit.....	14
Genetics Units.....	14
Tissue Culture Unit.....	15
Transparent Chamber Unit.....	16
Virus Oncology Unit.....	16
Cytology Unit.....	16
Leukemia Unit.....	17
Cell Physiology Unit.....	17
Biophysics Section.....	18
Biologic Effects of Ionizing Radiations Unit.....	18
Ionizing Radiations in the Multimillion Volt Range Unit....	18

	Page
Ultraviolet Radiations Unit.....	19
Internal Ionizing Radiations Unit.....	19
Radiohistology and Radiocytology Unit.....	19
Physical Analysis of Tissue Compounds Unit.....	19
Physico-Chemical Radiobiology Unit.....	20
Instrumentation Unit.....	20
Chemotherapy Section.....	20
Organic Chemistry Unit.....	20
Biochemistry Unit.....	22
Cytology Unit.....	23
Pharmacology Unit.....	23
Endocrinology Section.....	24
Pathology Section.....	25
Gastrointestinal Pathology Unit.....	26
Biochemistry Unit.....	26
Endocrine Pathology Unit.....	27
Geographic Pathology Unit.....	28
Gynecologic Pathology Unit.....	28
Short Term Tissue Culture Unit.....	29
Urologic Pathology Unit.....	29
Hematologic Pathology Unit.....	30
Veterinary Pathology Unit.....	30
Pathological Technology Unit.....	30
Laboratory of Experimental Oncology (San Francisco).....	31
Clinical Research Unit, Baltimore.....	32
Tumor Service, Baltimore.....	33
Epidemiology Section.....	33
Cancerigenic Research Laboratory, Georgetown Hospital.....	34
Editorial Office.....	34
Cancer Control Branch.....	35
Field Investigation Section.....	37
State Aid Section.....	38
Cancerigenic Studies Section.....	39
Public Health Nursing Section.....	41
Training and Project Grants Section.....	42
Professional Training Program.....	42
Grants and Fellowships Branch.....	42

## Introduction

Cancer research in the Institute's laboratories and cooperating clinics, cancer control activities, and support of research in non-Federal institutions have progressed steadily during the past year. The program of clinical investigation, expanded in 1949-50, continued to provide cancer patients with the latest benefits of medical science while permitting bedside study of the disease.

During the fiscal year ending June 30, 1951, the National Cancer Institute had approximately 60 units with an average staff of just over 500 (including research fellows). The appropriation for the year was \$20,086,000. This included \$5,000,000 for liquidation of construction grants already approved, \$3,353,000 for research grants, \$4,149,000 for State cancer control and special project grants, \$2,040,000 for teaching grants and \$875,000 for fellowships and clinical traineeships.

### TECHNICAL SERVICES Gilcin F. Meadors, Chief

The Office of Technical Services functions largely through three sections, Biometrics, Documentation, and Information, which render specialized services to the various sections of the Institute. It also recruits commissioned personnel for work in the cancer field and gives administrative supervision to such personnel while in training. The chief of the office maintains liaison with and attends meetings of the National Research Council's Committee on Cancer Diagnosis and Therapy. The main activities of the three sections are given below.

### BIOMETRICS SECTION Harold F. Dorn, Acting Chief

This section renders consultative services to and conducts studies in cooperation with other sections of the National Cancer Institute and outside agencies in addition to having major responsibility for a number of projects in the field of biometrics. Activities of the past year included:

Cooperation with the American Cancer Society and other organizations in studying the existing tumor nomenclature and preparing a statistical code and classification. This will be published soon by the American Cancer Society.

Development, in cooperation with a committee of the American College of Surgeons, of a proposed set of rules and procedures for tabulating the end results of cancer therapy.



Tabulation and analysis of the data collected in the survey of morbidity from cancer conducted in 10 cities. Reports on Atlanta, Georgia, and San Francisco, California, were published.

Initiation of a State-wide survey of morbidity from cancer in Iowa (in cooperation with Iowa State Department of Health). This is the first State-wide survey of this type ever to be conducted. It will provide data on a rural area, in contrast to the studies already made of metropolitan areas.

Preparation of plans for a study of the cost of cancer therapy (hospitalization and physicians' services) in the District of Columbia.

A number of special investigations in statistical methodology applicable to problems in cancer research.

Assistance to NCI, NIH, and outside experimenters in the design and interpretation of experiments.

Cooperation with the NCI Pathology Section in developing plans for an investigation of the etiological factors in cancer of the cervix in Jewish and non-Jewish women.

Assistance to the National Office of Vital Statistics (a) in preparing a special report on cancer mortality, published as Vol. 32 Vital Statistics-Special Reports; (b) in coding the occupation and industry on official death certificates for 1950. The latter data are for use in a study of mortality from cancer by occupation and industry.

Revision of a manual of instructions for operating a cancer register in a county health department.

Development of a procedure for determining the number of cancer diagnostic and treatment clinics which can be economically supported in a State. (Cooperative project with the Cancer Control Branch and The American College of Surgeons.)

Study of cancer facilities and treatment of cancer patients in Monmouth County, New Jersey (conducted at the request of the Monmouth County Medical Society and the local division of the American Cancer Society.)

Consultation services to State health departments in the field of statistics.

DOCUMENTATION SECTION  
E. F. Hoffman, Chief

This section is developing a system for classifying and indexing cancer literature which will make it possible to assemble quickly related abstracts

and references both from the pool and library resources and to reproduce copies for prompt distribution to scientists both in the Institute and elsewhere. To date approximately 10,000 references principally of cancer diagnostic or "cancer test" significance have been recorded or cut on I.B.M. cards and cross-indexed in the files; about 1,000 articles have been photostated and 100 have been translated and copied on hectograph plates; 2,000 full reference texts relating principally to cancer diagnosis or industrial cancer, and 250 abstracts have been placed in the document file pool. About 1,000 copies of the 2,000 reference texts have been prepared for distribution to outside scientists.

About 26,500 numbered basic code items of a chemical, physical and anatomic phylogenetic or static nature have been developed and are being used as needed to label each reference.

A complete up-to-date bibliography on Cytology in Cancer Diagnosis will be published soon. This will demonstrate the possibilities of the documentation service and be tangible evidence of the wealth of material stored up by this system to date.

CANCER REPORTS SECTION  
James F. Kieley, Acting Chief

The Cancer Reports Section is the office of the Institute through which all information activities are conducted and information materials are processed and distributed. Intramurally it is a service office, planning public information programs, preparing or assisting in the preparation of all Institute information materials, and handling all clearance procedures except on scientific papers, most of which are cleared through the office of the Acting Scientific Editor. It receives and answers all public inquiries and serves as a reference and information office for the staff.

CANCER RESEARCH BRANCH

The Cancer Research Branch is responsible for developing and carrying out the Institute's program of laboratory, clinical, and epidemiological research. In the year under review, this branch was composed of eight sections (biochemistry, biology, biophysics, chemotherapy, endocrinology, pathology, epidemiology, and the Laboratory of Experimental Oncology located in San Francisco), the Clinical Research Unit and a Tumor Service at the U. S. Marine Hospital, Baltimore, and the Cancerigenic Research Laboratory located at Georgetown Hospital, Washington. Each section is administered by its own chief, and, generally, the section is divided into a number of units, each under the supervision of a specialist in a particular field of research. Coordination of practices and policies is effected through frequent staff conferences of section chiefs with the Director of the Institute and members of his administrative staff. The various approaches to the cancer problem and the multiplicity of studies conducted by the research staff are reflected in the brief statements on the work of the individual sections and units which follow.

About one-third of the reports of the Institute's research are published in the bimonthly Journal of the National Cancer Institute, 1000 copies of which are made available without charge to scientific institutions and investigators throughout the United States and foreign countries. Approximately 120 papers by staff members were published in outside journals last year.

BIOCHEMISTRY SECTION  
J. P. Greenstein, Chief

Activities conducted by staff members working directly under the chief's office included the application of chromatographic methods to the analysis and separation of protein mixtures; the development of a high precision interference refractometer which automatically records changes in concentrations of various materials in the mixtures studied; a number of metabolic studies (the limitations of this report do not permit elaboration of these); and synthesis and study of the phosphorylated derivatives of pyridoxine. The analytical laboratory has examined 950 samples of materials and performed some 3,300 tests on these samples. Other services included the construction and/or assembling of scientific apparatus, the adaptation and application of apparatus, and the adaptation of analytical methods to the Section's studies.

The Enzymes and Metabolism Unit is chiefly concerned with investigation of the chemical and enzymatic reactions of proteins, peptides, amino acids, and allied compounds. The following projects were in progress or completed during the year:

A study of the complex formation of cobalt with several peptides and investigation of the enzymatic susceptibility of such complexes.

A comparison of the association constants of cobalt complexes of glycine with those of several dipeptides, employing a new graphical method of calculation.

Continuation of the studies of the enzymatic hydrolysis of the peptides of cystine.

Investigation of the activation of desoxyribonuclease by divalent metal ions. This study revealed a much wider range of activating ions than had been previously described, and the effect of ions derived from the glass vessels employed was shown to be negligible.

Studies of the chemical and enzymatic hydrolysis of diglycine, triglycine, and tetraglycine.

Study of the asymmetric enzymatic hydrolysis of carbobenzoxy-DL-glutamic acid, and resolution of DL-glutamic acid by this means.



Resolution of  $\beta$ -L proline by asymmetric enzymatic hydrolysis of the amide. This procedure employed a novel method of separating the products of the enzymatic reaction based on carbobenzoxylation of the mixture.

Purification of the hog kidney acylase employed in the amino acid resolution procedure, and study of its specificity. A separate enzyme hydrolyzing acyl aspartic acid was prepared and a low activity toward acyl-D-amino acids was demonstrated for the first time.

Investigation of the optical purity of amino acids resolved by the enzymatic method, as well as those obtained from commercial sources, employing specific amino acid oxidases. A high order of purity for the enzymatically resolved isomers was demonstrated.

Study of the alkali catalyzed ring closure of pyruvoyl glycine and demonstration of a possible biosynthetic pathway of pyrrole rings.

Studies of the catheptic activity of the nuclei of various normal and neoplastic tissues.

Investigation of chemical factors in the stability of the virus of Rous chicken sarcoma (in collaboration with the Biology Section).

The Clinical Biochemical Research Unit conducts both fundamental and clinical cancer research. Studies on clinical problems are carried out at the Warwick Clinic, utilizing patients available at this clinic and several hospitals in the Metropolitan Area. These studies are related to those of a more fundamental nature conducted at the National Cancer Institute.

The following projects were either under way or completed during the fiscal year 1950-51.

Studies on transamination involving glutamine.

Preparation of  $\alpha$ -keto acids using enzymes.

Preparation and study of the optically active keto analogues of allo-isoleucine and isoleucine.

Development of a simple enzymatic procedure for the separate determination of aspartic and glutamic acids.

Study of a unique biological decarboxylation reaction whereby aspartic acid is decarboxylated to  $\alpha$ -alanine.

Investigation of the nature and biological reaction of the phosphorylated derivatives of vitamin B<sub>6</sub>.

Study of the relationship between vitamin B<sub>6</sub> deficiency and enzymatic activity.

Investigation of the configuration of synthetic  $\alpha$ -amino-tricarballic acid and of the metabolism of this amino acid.

Study of the oxidative metabolism of a series of  $\alpha$ -hydroxy acids.

Study of the nature of the catalase inhibitor isolated from tumors and investigation on its mode of action.

Application of electrophoretic techniques to clinical leukemia.

Study of several enzymatic activities (including lactic dehydrogenase) in the serum of patients with cancer and other diseases.

Development of a high precision method for the evaluation of sedimentation constants of purified proteins in the ultracentrifuge.

Study of the physicochemical properties of the hemoglobin of patients with sickle cell anemia.

Study of the hydration of proteins by ultracentrifugal techniques.

Diffusion studies of optically active and racemic amino acids.

The Cytochemistry Unit studies conducted by this unit are directed at obtaining a greater understanding of the basic nature of subcellular and macromolecular mechanisms of normal and malignant cells. Since a preponderant proportion of the enzyme activity, particularly that associated with oxidative metabolism, takes place in the cytoplasm of cells, the studies have dealt primarily with cytoplasmic particulates. One approach has been through efforts to improve the fractionation procedures for isolating the various cytoplasmic entities.

Chromatography. Studies of the chromatographic technique of fractionation show that this method is applicable to macromolecules and larger subcellular particulates including viruses and microsomes, as well as the microscopically visible mitochondria of cells.

Studies of subcellular particulates of tumors led to the observation that oxidative enzymes can be inhibited or enhanced by common salt, depending upon its concentration.

Ascites tumors. Ascites tumors, which grow as free tumor cells when implanted intraperitoneally in mice, are being used for studies comparing the

enzymic behavior of the material in the form of intact cells, tissue slices, homogenates, and isolated particulates.

Particulate implants. Studies with melanoma fractions failed to confirm reports that certain subcellular particulates are capable of self-reproduction and exert nongenetic influences on the destiny of the cell and its behavior.

Ionic balance. Work has been initiated to study the sodium potassium balance of subcellular particles and its implications in tumor metabolism. Quantitative measurements are being made by flame spectrophotometer techniques.

Pigment formation in melanoma tumors. A long-term experiment designed to obtain information on the cellular inheritance of melanotic and amelanotic mitochondria in the S91 melanomas was continued.

Mitochondrial metabolism. Studies in this field included (a) morphologic and enzymatic characterizations of the mitochondria from melanomas, hepatoma, and normal liver; (b) the role of electrolytes and non-ionic substances (e.g., polyvinylpyrrolidone) in preserving the morphology and enzymatic integrity of isolated mitochondria; (c) the role of specific cellular enzymes in the vital staining of mitochondria.

Electrometric potential studies. Efforts were made to develop a technique whereby oxidation-reduction potentials and pH values could be recorded of liquid systems placed in modified Warburg vessels. From results obtained with dopa at pH 7.2 it seems to be possible to detect changes at least as small as 50 millivolts in biological systems such as tissue homogenates, bacteria, or alga suspensions.

Penichromin isolation and action. Crude penichromin was prepared from cultures of Penicillium notatum and fractionated by chemical methods. Two colored substances were detected, one in about five times the concentration of the other. Further work is being done on methods of fractionation and purification. Assay methods for biological activity of the penichromin fractions were worked out using mouse liver and hepatoma homogenates, or fractions thereof, e.g., mitochondria.

Photosynthesis. In collaboration with Professor Otto Warburg, Director of the Kaiser Wilhelm Institute of Cell Physiology, Berlin, significant advances were made in the understanding of the energy transformation process in carbon dioxide fixation. These studies are of interest in connection with nutrition, food supply, natural energy resources, and biochemical energy exchanges common to both photosynthesis and cancer.

The work of the Metabolism and Growth Unit centered on three main points:

Study of the metabolism of p-dimethylaminoazobenzene (DAB), its rate of absorption and excretion and if possible, the determination of the binding between DAB and liver proteins which eventually leads to primary hepatic tumors.

Study of the metabolism of  $N^{15}$  labeled as well as  $C^{14}$  labeled amino acids.

Study of the glyconeogenic properties of some of the essential amino acids both in normal and tumor-bearing animals.

Study of  $N^{15}$  labeled p-dimethylaminoazobenzene fed to normal and tumor-bearing rats showed that 90 percent of the chemical is eliminated in 72 hours. A small amount is found in the liver, reaching a maximum concentration in 5 to 6 weeks. The evidence indicates that the chemical forms a bound dye with some of the liver proteins; the dye-protein complex is then split at the azo linkage of the dye, leaving a phenylenediamine protein dye complex. Further work to determine the exact nature of this linkage is in progress.

Valine labeled in the methyl groups with  $C^{13}$  and in the Beta carbon with  $C^{13}$  was prepared and resolved. It has been demonstrated that three of the five carbon atoms of valine are converted to glycogen and the mechanism of this conversion was demonstrated.

Studies were made of the conversion of L-valine to  $CO_2$ , glucose, and glycogen; of the conversion of D-valine to glycogen; and a comparative study was made of the conversion of L-valine to carbon dioxide and glycogen in normal animals and in animals receiving p-dimethylaminoazobenzene.

A new method of synthesizing  $\omega$ -benzoylaminovaleric acid was developed as a byproduct of an unsuccessful attempt to synthesize  $\alpha$ -keto- $\omega$ -aminocaproic acid.

Marked differences were found in the metabolism of intravenously administered  $N^{15}$  labeled D- and L-phenylalanine in the rat. Similar differences were found for L- and D-valine.

Gross metabolism studies of  $C^{14}$  labeled histidine in normal and tumor-bearing rats indicate that the isotope is distributed through most of the tissues of the body, and that with the exception of liver and plasma, the tissues of the cancer-bearing rat incorporate up to three times the amount of histidine incorporated by the tissue of the normal animal. This seems to indicate that there is a systemic stimulation of the metabolism of histidine in the host animal which is not supplied by tissue histidine.

Distribution of radioactivity in the individual amino acids after  $C^{14}$  histidine administration showed a considerable enrichment in methionine and serine.



Normal liver tissue was found to contain a higher concentration of ribonucleic acid nucleotides than liver tumor tissue, with the exception of cytidylic acid which was about the same in both tissues.

Studies in the Nutrition Unit included the following:

Studies on relation of molecular structure of chemicals to carcinogenesis. This is part of a program to discover the mechanism of 2-acetylaminofluorene (2-AAF) carcinogenesis. A single chemical group change is tested for its effect on carcinogenicity. Thus, substituting an acetyl group for a hydrogen in the AAF molecule, to form diacetylaminofluorene, resulted in a more potent liver-cancer-producing-chemical. The female sex hormone decreased its activity almost completely.

Chemical and stable isotopic tracer studies on the mechanism of carcinogenesis by 2-acetylaminofluorene. Comparison of the distribution in the rat of isotopic nitrogen and diazotizable nitrogen following the oral administration of  $N^{15}$  labeled and unlabeled 2-acetylaminofluorene (2-AAFN<sup>15</sup>), showed that 2-AAFN<sup>15</sup> has a wide distribution but a very transient stay in the tissues of the rat qualitatively similar to diazotizable nitrogen in 2-AAF. The chief pathway of excretion is the urine although a considerable portion of  $N^{15}$  labeled material is found in the feces, which is not detected by diazotization.

Radioisotopic tracer studies on the mechanism of carcinogenesis by 2-AAF. Activities included: studies on the properties of metabolites of 2-AAF-9-C<sup>14</sup> found in the feces and urine of rats; work on paper partition chromatography of bile from rats fed 2-AAF-9-C<sup>14</sup> in order to separate and isolate the metabolites present; studies of the absorption of 2-AAF through the stomach wall; synthesis of and improvement in the synthesis of chemicals known or suspected to be carcinogenic.

Production of transplantable thyroid tumors. Thyroid tumors were produced in C3H mice by hormonal imbalance establishing for the first time the principle that prolonged stimulation of a tissue by the animal's own secretions can result in cancer. These transplantable tumors provide material for many further studies.

Nutritional studies. The effects of pyridoxine deficiency were studied in five strains of mice - C3H, A, C57, C58, and dba. Survival time varied greatly. Pyridoxine deficiency compatible with long survival did not prevent spontaneous mammary tumors, lung tumors, or leukemia.

BIOLOGY SECTION  
H. B. Andervont, Chief

The work of the Biological Studies Unit is directed at ascertaining the relative importance of the many factors involved in the occurrence and growth of all types of tumors in experimental animals. Results:

Administration of atabrine, inositol or paraaminobenzoic acid increased the mean age at which mammary tumors appeared in mice but did not influence the tumor incidence.

The microsome fractions from normal and neoplastic mouse tissues and guinea pig tissues were injected into rabbits and the resultant antisera used in complement fixation studies. It was found that the microsome fractions are antigenic and react more strongly with antisera against the homologous fractions. No tumor-specific antigen was detected.

Trypan blue injected into adult mice to determine whether the treatment increased their susceptibility to infection by the mammary tumor agent gave negative results but showed that adult mice inoculated with the agent did not harbor it in their spleens.

Methylcholanthrene induced tumors in fetal tissue when the two substances were implanted together into adult tissues. When they were placed in the liver, spleen and testis, tumors were induced in the fetal tissue despite the native resistance of these organs to the carcinogen.

Tumors developed both in ovaries from young mice and 2-month-old mice after they were transplanted into the spleens of castrate hosts.

Administration of urethane to pregnant mice during the late stage of gestation induced pulmonary tumors in the offspring, even when they were removed from the uterus of their mother only a few hours after she received the urethane.

The co-carcinogenic action of croton oil for methylcholanthrene was shown to be effective when the carcinogen was injected into the muscle. Sarcomas were induced.

The application of croton oil to the skin after treatment with methylcholanthrene led to the production of papillomas only. Croton oil alone did not induce papillomas.

Hybrid females, derived from two inbred strains show a high incidence of mammary tumors in the absence of a demonstrable mammary tumor agent. On the assumption that the mothers of the hybrids may carry an agent of very low activity, the mothers were exposed to X-radiation or estrogenic stimulation to activate the weak agent. Neither attempt was successful.

It was found that, in a few instances, the mothers did develop mammary tumors and carried the agent. Available evidence indicated they had acquired the agent from their mates which came from a high breast-tumor strain and carried the agent.

It was found that high dilutions of the mammary tumor agent could infect susceptible mice without producing tumors in them, but their offspring developed tumors. This indicates that in making quantitative studies on the agent, offspring from mice inoculated with the agent should be used.

Wild house mice were shown to be susceptible to the agent from inbred mice and, furthermore, could transmit it through several generations. Foster nursing experiments showed that wild house mice may carry the mammary tumor agent, despite a low incidence of mammary tumors.

The incidence of spontaneous hepatomas in different groups of males from a certain inbred strain varied between 12 and 55 percent. This wide variation in the incidence of hepatomas in untreated mice indicates the necessity of adequate controls in any effort to establish the influence of diet or hormones in the occurrence of these tumors.

Studies on cell adaptation by the Cell Adaptation Unit were started in the belief that some types of the carcinogenic process are related to the process of cell adaptation to unfavorable environments. Since such studies are best performed with single-celled organisms, bacteria were used in most experiments. Results:

A rhythmic exposure of serial culture populations of bacteria to unfavorable conditions resulted in the adaptation of the organisms to the unfavorable environment.

With the rhythmic exposure of bacteria to high temperatures, the occasional emergence of new types has been observed. These new forms have been studied intensively and the evidence thus far indicates that they are not contaminants.

The studies of the Cell Chemistry Unit deal with the relation between cell structure and cell chemistry to ascertain whether specific cell structures are intimately concerned in the process of carcinogenesis. The most significant findings were as follows:

The demonstration that a large proportion of the total mass of liver or hepatoma mitochondria consists of soluble proteins that are released when the mitochondrial membranes are disrupted, and that the most prominent protein of normal liver mitochondria is apparently absent from hepatoma mitochondria.

The demonstration that mitochondria are the site within the cell of the aerobic synthesis of the high-energy phosphate compound, adenosinetriphosphate. This finding is of particular interest since adenosinetriphosphate, in turn, supplies energy for a number of important biochemical reactions within the cell.

The demonstration that, under certain conditions, the desoxyribose nucleic acid of thymus and liver nuclei is not associated with structures

comparable to the so-called "intermitotic chromosomes" but is colloiddally dispersed in the form of very small particles.

Studies in the Developmental Physiology Unit are concerned with the relationship between differentiation and carcinogenesis. Using the early mouse embryo, studies were made of the growth and differentiative behavior of the early embryonic shield in flask culture and in the anterior chamber of the eye. Results:

Hybrid embryos were found to be a more satisfactory tool than those from an inbred strain.

In flask cultures, clusters of 4-8 shields grew rapidly and reliably, whereas single shields frequently deteriorated.

Exposing cluster cultures to various substances which have been reported to affect growth and differentiation gave negative results. This raises the question whether differentiation is incited more readily in an organized cell mass than in the isolated cell.

Studies in the Gastric Cancer and Tumor Immunity Unit are concerned with (1) conditions which may be important in the development of gastric cancer and (2) the fundamental factors involved in tumor immunity and tissue transplantation. Results:

Observations on dogs having a permanent gastric fistula show that the method of treating gastric ulcer in humans by surgical implantation of jejunal pedicle grafts may be unsound.

A transplantable tumor grown in a hybrid host undergoes a consistent and definite adaptive change which is permanent.

Foster nursing of the hosts had a major effect upon the growth rate of a transplanted tumor.

A tumor was found which grows readily in two inbred strains of mice. Red blood cells from one strain immunize the other against the growth of the tumor. When the cells are laked in water, or by freezing, or destroyed by mild sonic vibrations, they do not immunize. It is highly significant, however, that washed cell membranes did elicit immunity.

The Genetics Unit studies the role of heredity in the occurrence of tumors. Results:

By the transplantation of susceptible and resistant lung tissue into a common host it was found that the action of the genes controlling susceptibility to pulmonary tumors in mice was localized in the lung tissue per se or was manifest through some general systemic physiology. Lung tumors arose only in the genetically susceptible tissues.



Further studies on linkage between specific known genes and the development of pulmonary tumors showed that the hairless gene was associated with a reduction in susceptibility to the spontaneous growths, whereas the pink-eye gene had no effect. Neither gene influenced the incidence of mammary tumors, liver tumors, or skin papillomas. The flexed-tail, shaker-2 and waved-2 genes were found to be associated with a reduction in susceptibility to spontaneous lung tumors, but none influenced the incidence of mammary or liver tumors. A gene which increased susceptibility to lung tumors also increased body weight, and those which decreased susceptibility to the tumor also decreased body weight.

A line of C3H mice deprived of the mammary tumor agent continued to show a high incidence of mammary tumors in breeding females, and further efforts to detect an agent in them (by foster nursing and hybridization) were unsuccessful.

Mammary tumors were induced in agent-free males by the administration of stilbestrol.

Strain hairless mice were painted with methylcholanthrene. All mice developed papillomas of which many progressed to carcinomas.

Untreated strain hairless mice showed a high incidence of spontaneous hemangio-endotheliomas.

Studies in the Tissue Culture Unit are concerned with the application of the techniques of tissue culture to problems of growth and cancer.

Nutrition Research. Procedures were developed for preparing and handling replicate cultures and for measuring proliferation in tissue cultures. The influence of inoculum size on proliferation of strain L cells was determined.

Both qualitative and quantitative comparisons were made of the growth of chick heart and strain L fibroblast planted as suspensions on Pyrex glass and perforated cellophane substrates.

Research on massive growth of tissues. Animal tissue cells were grown on three dimensional substrates.

Carcinogenesis research. Hepatoma, thyreocarcinoma, and melanoma tissue of the strain C3H mouse were cultured in vitro in conjunction with three other lines of research. All three tissues produced sarcomas when injected.

Endocrinology research. (In collaboration with the Endocrinology Section). The rat anterior pituitary tissue was maintained in vitro for 164 days, but gonadotrophic activity decreased in the supernatant fluids. Methods for measuring gonadotrophic activity in the supernatant fluid were developed.

Research on embryo extract. The addition of small amounts of hyaluronidase, followed by ultracentrifugation (30,000 X gravity) for 90 minutes, permitted bacteriological filtration. The effect of this filtrate on tissue culture

proliferation was tested. The level of proliferation was nearly as high as in cultures on which untreated embryo extract from the same original pooled lot was used. This treatment makes possible the nonsterile extraction, preparation, and handling of large lots of embryo extract for standardization.

The technique developed in the Transparent Chamber Unit is used to advance understanding of problems related to cancer. Results:

Studies of vascular reactions of mice to fibroblasts treated in vitro with methylcholanthrene suggested the possibility that growth in vitro for a prolonged period of time in heterologous media may so alter the characteristics of the cells as to mask their carcinogenic potencies.

A technique was designed for measuring the blood pressure in arteries supplying a tumor. Peripheral hypotension induced by histamine resulted in tumor damage.

A method was devised for graphic recording of heart rates of unanesthetized mice.

The response of a transplanted mouse mammary adenocarcinoma to local irradiation was determined. Regression seemed to be primarily a direct response of the tumor cells, and the blood supply decreased markedly as a response to tumor cell damage.

The object of the work of the Virus Oncology Unit is to obtain the agent of a chicken tumor in the pure state so that it may be used in basic studies on the nature of the agent and its carcinogenic action. Results:

It was found that citrate ions exert a stabilizing influence on the biologic activity of the agent. This makes possible the fractionation of large batches of tumor extracts.

The work this year has solved a major problem, namely, preservation of the activity of the highly labile agent, and set the stage for controlled systematic investigations on the purification of the agent.

The work of the Cytology Unit is concerned with comparisons of the finer structures of normal and malignant cells to determine whether the change to malignancy is reflected in these structures.

In collaboration with the Nutrition Unit, Biochemistry Section, malignant thyroid carcinomas were produced.

Detailed studies were made of cells of stomach, small and large intestine, salivary glands, liver, pancreas, and kidney. Many new and highly significant



structural modifications of epithelial cell types were found which were hitherto unsuspected or debatable.

The Leukemia Unit studies the biological aspects of leukemia in experimental animals with special emphasis on agents of possible therapeutic value.

Studies on folic acid antagonists produced the following results:

A transplantable lymphoid leukemia of the mouse became resistant to the effect of three of these antagonists. In part, the tumor became dependent upon the antagonist for optimal growth and retained this characteristic through many animal passages.

Two compounds were found which reversed this optimal growth-promoting capacity of the antagonists.

Experimental evidence indicated that spontaneous mutations occur in a population of leukemic cells and the antagonists act as selective agents.

These studies suggest that treatment should involve the use of several agents as early as possible and at the highest tolerable dosage.

Total thymectomy reduces the incidence of leukemia in mice. By using mice of differing genetic constitutions for transplantation of thymic tissues it was shown that the implanted thymus becomes reconstituted with lymphocytes of the host, and these lymphocytes become the malignant cells. Apparently the reticuloendothelial elements of the thymus influence the malignant transformation.

A transplantable leukemia was found to be contaminated by a virus which could be separated from the malignant cells.

A claim that frozen mammary tumor tissue of mice contains a virus which produces tumors rapidly at the site of inoculation was disproved. By using animals of differing genetic constitutions it was shown that the tumors arising at the site of inoculation of the so-called "virus" were derived from cells in the inoculum and not from the animal receiving the virus. Thus, the frozen tumor tissue produced tumors because of the presence of living cells rather than a "virus."

Studies of the Cell Physiology Unit on frog kidney adenocarcinoma have shown that transplanted strains can be established which up to now have grown only in frogs. The tumor has also been transmitted to frogs by a cell free inoculum. The tumor is being propagated in tissue culture.

Spontaneous tumors have occurred in salamanders imported from Japan. The biological characteristics of these tumors are under study.

Further studies on cytological effects of Beta-ray damage showed that a certain amount of these rays damage the nuclear membrane first, the nucleus next and the cytoplasm subsequently. Thus, the nucleus and cytoplasm exhibit qualitative differences in reactions to these rays.

BIOPHYSICS SECTION  
Egon Lorenz, Chief

This section is at present engaged in studies of the effects of ionizing radiations, the physical properties of cell tissue constituents, tracer studies with radioactive isotopes, metabolism studies to determine the fate of amino acids and carcinogenic agents in normal and tumor-bearing animals, and studies of ultraviolet radiations. Some of the work is done in cooperation with the Argonne National Laboratory, Chicago, Ill., and the Atomic Energy Commission which bear a share of the costs. Some of the units of this section are located at the University of California, Massachusetts Institute of Technology, and Princeton University. The main findings of the various studies are listed below.

Biologic Effects of Ionizing Radiations Unit (In collaboration with the Argonne National Laboratory, Chicago, Ill.).- A comparison of the LD 50-30 days in mice exposed to equal doses of radiations with different specific ionization showed that the radiation with high specific ionization (alpha radiation) is somewhat more effective than x-radiation. The difference probably can be accounted for by the nonuniform distribution in the body of the radiation of high specific ionization.

Studies of the effects of starvation and hyperoxia, respectively, during irradiation showed that the sensitivity of a transplanted mouse lymphosarcoma to ionizing radiations could be enhanced by these means.

Injections of homologous bone marrow intraperitoneally and especially intravenously or post-irradiation gave very good protection from the acute lethal irradiation syndrome in IAF<sub>1</sub> mice and inbred guinea pigs exposed to total doses which are 100 percent lethal without bone marrow injections.

Ionizing Radiations in the Multimillion Volt Range Unit (Work located at University of California Medical School, San Francisco, and the Massachusetts Institute of Technology, Cambridge, Mass.).- Measurements on scattering of secondary electrons produced by gamma radiation in materials of various atomic numbers gave important information on the construction of efficient gamma radiation detectors.

The "effective" atomic number of bones was determined for various types of radiations by measuring back-scattered electron intensity. It was found to be  $Z = 11.5/0.5$ . This value is in good agreement with the one obtained from the chemical composition of bone.

A positive ion source was built giving beam currents up to 200 microamps of hydrogen ions containing approximately 10 percent of protons.

A general focusing theory of ion focusing in Van de Graaff accelerators was developed and applies successfully to an existing machine.

Photoreactivation following injury by ultraviolet radiation was studied extensively in the Ultraviolet Radiations Unit. It could be shown that ultraviolet radiation, as far as cell division is concerned, acts on the nucleus. Photorecovery due to visible radiation is a property of the egg cytoplasm. It was further shown that photorecovery exists also in a vertebrate animal indicating that this is a phenomenon of general occurrence in living organisms.

The Internal Ionizing Radiations Unit has made a survey of the functions of a large number of transplantable mouse thyroid tumors. A considerable number of tumors concentrate  $^{131}\text{I}$  but only a small fraction of these concentrate  $^{131}\text{I}$  per mg. of tumor tissue to an extent greater than 1 percent of that concentrated by the thyroid of the same host. These tumors are similar to those in humans in this respect. Preliminary studies on the effect of hypophysectomy on function of mouse thyroid and thyroid tumor indicate that while the thyroid is dependent upon the intact pituitary for the concentration of  $^{131}\text{I}$ , the tumor is not.

Further studies in the Radiohistology and Radiocytology Unit on irradiation injury of mouse testes show that the over-all picture can be explained on the basis of the selective sensitivity of a single stage of spermatogenesis, that of mitosis of spermatogonia. The degree of inhibition is a function of the dose.

Determination of the desoxyribose nucleic acid content of the nuclei of normal thymocytes and two lymphoid tumors used in a study of irradiation treatment of these tumors revealed that the nuclei of the cells of one tumor contained twice as much desoxyribose nucleic acid as do the cells of the second tumor and of normal thymocytes. This has been found to be correlated with the total number of chromosomes per nucleus.

An evaluation of the usefulness of tetrazolium salts for the histochemical localization of sites of dehydrogenase activity showed that these salts can be used as indicators of succinic dehydrogenase activity under certain conditions. The chemical finding that succinic dehydrogenase is localized in or on mitochondria can be confirmed by this histochemical method.

Physical Analysis of Tissue Compounds Unit investigated the pH of tumors under different experimental conditions. Large acid effects were observed in some tumors when glucose was injected intraperitoneally. No such acid shifts were found in normal tissue.



In electron microscope studies, remarkable fibrous structures of cysteine were found. The appearance of fibrous structures could be traced to contamination with minute amounts of copper. This finding is highly significant in the interpretation of biologic material with the electron microscope.

Physico-Chemical Radiobiology Unit used the mass spectrometer in a study of the respiratory metabolism of normal and irradiated animals. In irradiated animals there is a sharp decrease in oxygen consumption. The respiratory quotient values obtained indicate waves of marked acid-base shifts in the blood buffer system of irradiated animals.

It was shown that unknown compounds could be identified in mass spectrometric analysis by making use of the Law of Graham which states that the effusion rate is inversely proportional to the square root of the molecular weight. Mixtures of as many as five compounds were analyzed and the molecular weight determined by this method.

A project is under way on the effects of x-irradiation of single cell systems. It was shown that water plays an important role in the irradiation death of bacteria. Water suspensions are 30 times as radiosensitive as vacuum dried bacterial preparations.

Some of the more important contributions of the Instrumentation Unit were the improvement of methods for the mass production of tissue culture flasks of various sizes by making rectangular bore tubing with uniform wall thickness; construction of Geiger Müller counters with a thin (0.1 mm.) tube of 1 mm. diameter leading into the interior of the counter; designing of micro instruments such as micro syringes, micro scalpels and micro blenders; working out methods to manufacture flat grooved glass disks by mass production methods; designing and making all-glass animal cage, automatic all-glass triple still, rotating camera support, rotating incubator, glass, and metal dialysers, paper strip pullers, and all-glass continuous extracting columns.

CHEMOTHERAPY SECTION  
M. J. Shears, Chief

The four units of the Chemotherapy Section are engaged in an integrated program directed at selecting, evaluating, and developing chemical agents which may be useful in treating cancer in human beings. About 3,000 compounds have been tested to date, of which approximately 400 have demonstrated the ability, when injected in a single near-lethal dose, to produce injury in sarcoma 37 in mice. The major emphasis of the Section's work is now being placed on more intensive study of certain of these tumor-damaging compounds. A limited amount of screening of new chemicals will be continued.

Organic Chemistry Unit studies of podophyllin and derivatives. Following the demonstration of the stereoisomerism of podophyllotoxin and picro-podophyllin, the structures of the other two potent compounds in the American

variety of May apple, i.e., alpha- and beta-apopicropodophyllin, were established. An older formula for alpha-apopicropodophyllin was confirmed; an older formula for the beta-isomer was disproved, and a new structure for the later proposed. A new compound, desmethyl-beta-apopicropodophyllin was prepared and its structure proved.

Inasmuch as water-soluble derivatives of podophyllotoxin would possess pharmacologic advantage, a number of such compounds were prepared, i.e., alpha-peltatin sulfate, beta-peltatin sulfate, podophyllotoxin isothiuronium bromide and podophyllotoxin succinate.

A species of podophyllum native to India (P. emodi) was fractionated in the hope that it might contain new compounds of biologic interest. It was found to contain about four times as much podophyllotoxin as does P. Peltatum, much less alpha- and beta-peltatin, and a considerable amount of a new crystalline compound which appears to be a d-glucoside of picropodophyllin.

Studies of colchicine and its analogs. Colchicine and its derivatives continue to be of major interest because of their ability to damage tumors at doses considerably below the lethal. Despite the large amount of work done on colchicine, its structure is still in doubt, and can be proved only by synthesis. The difficulty surrounding this problem is indicated by the fact that perhaps 20 steps will be required for its synthesis. Considerable progress has been made, however, and several intermediates in the colchicine series containing the 6-membered A-ring fused to a 7-membered B-ring, with the proper substituents on each, have been synthesized. The 7-membered C-ring can now be added.

Since it was shown that colchicine amide has a higher maximum tolerated dose than colchicine, with a not much greater minimum effective dose, a series of 14 N-substituted colchicinamides was prepared. All showed activity against sarcoma 37.

Studies of phenazine derivatives. A large number of phenazines with different substituents have been synthesized and screened against sarcoma 37 for activity. A new type of phenazine (di-N-oxides) has also been shown to be active.

Two fundamental contributions to organic chemistry have been made in the course of this investigation: (1) the finding that the usually non-reactive chlorine atom in the 2 position of phenazine can be made reactive by converting the nitrogen atoms of the phenazine to N-oxide and, (2) that in the Waterman-Vivian ring closure reaction of diphenylamines to form phenazines, where substitution is such that 2 possible phenazines could be found, both phenazines can be isolated. The practical value of these findings is the increased accessibility of new phenazine derivatives.

Studies of organic arsenicals. Several new arsenicals of types known to produce damage in sarcoma 37 were synthesized.

• Collaborative clinical studies. Two different preparations of beta-peltatin for oral administration were developed for clinical study. Suitable preparations of this compound for intravenous administration are being worked on.

Materials for screening. 271 new compounds and plants have either been synthesized or procured for the screening program.

Report on "Compounds which have been tested for carcinogenic Activity." The first edition of this report was published in 1941. A second, enlarged, edition will be available soon.

Biochemistry Unit screening program. The therapeutic index for a single injection was determined for 340 tumor-damaging compounds by ascertaining the range between the maximum tolerated dose and the minimum effective dose. Most of the compounds showed potency only at near-lethal doses. About 90 percent has a therapeutic index of less than two.

Fractionation of podophyllin. It has been shown that the podophyllinic acid fraction of podophyllin has an ability to damage tumors which cannot be entirely accounted for by the presence of podophyllotoxin, alpha- or beta-peltatin. Chromatographic study of this crude fraction showed that over half of its activity is due to an unknown material, which behaves biologically in a manner similar to the previously known constituents.

Mechanism studies. Nine different colchicine derivatives, respectively, were shown to cause a decrease in the cytochrome oxidase activity of the tumor when injected into animals bearing either sarcoma 37 or lymphoma L 2.

Studies of the role of the adrenals in the effects of podophyllotoxin on the hematopoietic system of rats showed that in adrenalectomized rats there was a significant leucopenia (mononuclear, polymorphonuclear, and eosinophilic) within 1 hour after injection. Adrenal damage was evident in the intact rats. Damage to bone marrow and lymphatic tissue was observed in both groups. These findings suggest that the action of podophyllotoxin on the hematopoietic system is a result both of a nonspecific stress phenomenon and of a specific cytotoxic action.

Using the developing chick embryo as a tool, a bioassay method was developed which makes it possible to determine the fate and distribution of podophyllotoxin in animals. By this method it was shown that podophyllotoxin injected into rats was converted within 2 hours to a form nontoxic to the chick embryo. In another set of experiments, in which animal carcasses and tissues were exposed to podophyllotoxin in vitro, a slower conversion of the drug into a nontoxic form occurred.



Cytology Unit microscopic study of treated tumors. Many specimens of sarcoma 37 have been examined histologically for cellular damage, following chemical treatment of the host. Such damage consisted of reversible and irreversible arrest of chromosomes in mitosis, intracellular edema, necrosis, pyknosis, distorted polar distribution of chromosomes, reduction or increase in reticulum, and formation of giant "tumor" cells that may be polynucleated or polymorphonuclear.

Cytology of sarcoma 37. A cytological study has been made of sarcoma 37, including minerals, mitochondria, Golgi apparatus, glycogen, sudanophilic fat, chromosomes, nucleus, reticulum, blood vascular endothelium, fixed histiocytes, and mast cells. The cytology of the tumor was studied by means of various techniques--microincineration, ultracentrifugation, and sections prepared from specimens using liquid air freezing-dehydration in vacuo. The cellular behavior of this tumor was studied from 1 hour up to 12 days after implantation in the thigh muscle.

Cytology of liver tumors. A study of liver tumors of rats and mice is being made. It has been found that the large conspicuous nucleoli characteristic of many types of malignant cells are in liver tumor cells apparently due to a moribund process, since such large nucleoli appear chiefly in cells that lack mitochondria and show evidence of alteration that leads to an early death.

Pharmacology Unit screening of naturally occurring substances for tumor-damaging capacity. Two of the 90 plant products screened for tumor-damaging capacity have yielded results which warrant more intensive study. Fractionation has been started to isolate and identify the active constituents involved.

Studies on the mechanism of action of bacterial polysaccharide from S. marcescens.

Eosinophil variations in the rat following injection of a tumor-necrotizing bacterial polysaccharide. Intraperitoneal injection of this agent in rats produced hematologic variations which indicate that it activates the pituitary-adrenal mechanism at least in part; and that this purified bacterial product is capable of eliciting a similar kind of alarm reaction as that following bacterial infections or the administration of crude bacterial extracts described in previous reports.

Site of the immunity response following administration of bacterial polysaccharide. Since one of the drawbacks to the clinical use of polysaccharide is the antibody response which follows the first injection and interferes with the effect of subsequent doses, it is important to determine the site of the antibody response in order to plan studies on ways to break down this immunity. An experiment directed at obtaining information concerning this problem has been completed.

Protection by cortisone against tumor-damaging action of S. marcescens polysaccharide.

Studies conducted on mice bearing 6-day old sarcoma 37 showed that the minimum dose of polysaccharide required to induce marked tumor damage was twice as large for mice which received an intra-peritoneal injection of cortisone 2 hours before the polysaccharide, as for mice which receive no cortisone injection.

Large Scale production of bacterial polysaccharide from S. marcescens.

Bacterial polysaccharide (P-25) has been brought to an advanced stage of purification. Step-by-step bioassays showed diminished toxicity without loss of potency. This material has been made available to numerous investigators throughout the country for a variety of researches.

#### ENDOCRINOLOGY SECTION

Roy Hertz, Chief

The Endocrinology Section is engaged in a coordinated clinical and experimental study of the relationship between the hormones and the cause and treatment of cancer.

The Section includes a Chemical Unit, a Bioassay Unit and a Clinical Unit, the latter at George Washington University Hospital. The activities of all the units are closely integrated and accordingly are reported together. The main accomplishments of the year are briefly summarized below.

After demonstrating that continuous intravenous and subcutaneous administration of a specially prepared form of water soluble estrogen brought about regression of breast and prostate cancer and that such dosages were well tolerated, an extended study was undertaken of the metabolism and fate of such estrogen dosage. An accurate measure of the enormous capacity of the body to inactivate estrogen has been made. The data permit the conclusion that about 25 percent of a large dose of estrogen is inactivated by the liver and at least an additional 40 percent is cleared by the kidney. Further attempts to increase the efficiency of a given dose are being made.

Treatment with progesterone has brought about regression in a number of cases of cancer of the cervix. Further observations are being made on related steroids. Initial studies on administration of progesterone by vaginal suppository indicate good absorption.

It has been shown that even massive doses of progesterone do not cause histidinuria. Accordingly the increase in urinary histidine in pregnancy is not attributable to progesterone, but probably depends on the elevated corticoid blood level.

Eighty steroid substances have been screened for anti-estrogenic activity in relation to progestational activity. Five showing little progestational activity but marked anti-estrogenic effect are being prepared for clinical trial.

Using chicks, studies on hormone-vitamin interrelationships have demonstrated that when estrogen-induced lipemia is inhibited by thyroxin, elevation of biotin activity in the serum usually seen in the estrogenized chick is also inhibited.

Amphenone "B" synthesized in this laboratory is the first nonsteroidal substance found to have a progesterone-like effect on the rabbit uterus. It also causes enlargement of both the adrenal and thyroid gland of the rat. Extensive biological studies are being conducted with this compound and a series of related substances now being synthesized by special electrochemical methods.

Biological tests of paraoxypropiofenone (PONE) have been made on parabiobiotic rats. The compound was found to be a very weak estrogen and an equally weak pituitary depressant.

Studies of the adrenal content of the "B" vitamins when the adrenal is activated by ACTH show that riboflavin, nicotinic acid, folic acid, and biotin remain constant when the adrenal is activated sufficiently to show maximal drops in vitamin C and cholesterol. These data emphasize the specificity of the reactivity of adrenal vitamin C to stress.

Experimental studies in the rat show that the atrophy of the prostate and of the breast induced by the removal of critically required trophic hormones, follows a highly reproducible curve with respect to time, and that this curve is not materially altered by thyroidectomy, adrenalectomy, caloric restriction, or the administration of such general catabolic agents as thyroxin or cortisone.

Other studies included the following: (1) metabolic studies on patients with hormone-producing tumors; (2) the nature of the antigonadotropic substance produced in the sera of animals subjected to prolonged treatment with pituitary gonadotropin; (3) the preparation of a potent concentrate of the antibiotin factor in egg albumen for use in biological studies; (4) the preparation of a large quantity of amphenone "B" and the synthesis of analogs of this chemical in an effort to improve on its activity and to correlate chemical structure with biological activity; (5) synthesis of an unsymmetrical pinacol by direct bimolecular reductions; (6) studies of the pinacol-indene reactions; (7) preparation of a number of new Mannich bases and the synthesis of a number of new Mannich pinacols.

PATHOLOGY SECTION  
H. L. Stewart, M.D., Chief

The Pathology Section studies the pathological aspects of the formation of tumors of various types. The work includes studies of histopathology,



histochemistry, chemistry, cytology, and physiology of normal, hyperplastic, and neoplastic tissues; also studies of endocrine and endemic factors in cancer. The Section serves as consultant and adviser in pathology to the research staff of the Institute and, upon request, to outside investigators. It administers a pathological technology laboratory which prepares histological slides for the entire Institute.

Gastrointestinal Pathology Unit. The following experiments are under way or completed:

Study of the pathologic anatomy of commonly used strains of experimental rats to determine the spontaneous occurrence of different lesions and their frequency.

Study of the effects of catechol on the gastrointestinal tract of mice. Catechol is a chemical isomer of dihydroquinone and resorcinol and is used commercially in solutions or ointments as an antiseptic for superficial wounds or burns and also as a preservative of canned foods.

Studies on mice bearing transplantable adenocarcinoma of the stomach showing morphologic changes of hypervolemia. These tumors are the first of nonendocrine origin known to produce hypervolemia in the host animal.

Study and histologic classification of gastric sarcomas in mice induced by methylcholanthrene.

Studies of the effects of external radiation on gastric mucosa in mice and the subsequent response of these mice to the feeding of carcinogens.

A number of experiments are in progress in efforts to produce gastric tumors and tumors of the colon.

Biochemistry Unit. The following projects were completed:

Induction of hepatic carcinoma and other lesions in rats by 4-dimethylaminobenzene-1-azo-1-naphthalene and their comparison with tumors produced by paradimethylaminoazobenzene.

Study of acid and alkaline phosphatase of serum and hepatic tissue of rats fed paradimethylaminoazobenzene in diet.

Toxicity study of 4-dimethylaminobenzene-1-azo-1-naphthalene. Three groups of 10 Osborn-Mendel rats were kept on 0.15, 0.3, and 0.6 percent azo dye in the diet. Average survival time was 19.6, 11.6, and 9.8 months respectively. Death was not directly referable to any lesion caused by the azo dye but presumably was due to increased susceptibility to bacterial invasions. Number of liver tumors produced in animals surviving after 12 months was

increasingly higher with higher dye concentration in the diet. Other projects under way included:

Attempts to induce lesions in the glandular stomach of C57 brown mice with 9, 10-dimethyl-1, 2-benzanthracene in Triton. Five forestomach tumors were found at 150 days.

Attempts to induce glandular stomach lesions in C57 brown mice with gallium citrate and methylcholanthrene.

Attempts to induce forestomach lesions in rats with 4-dimethylaminobenzene-1-azo-2-naphthalene.

Toxicity study of 4-dimethylaminobenzene-1-azo-2-naphthalene.

Study of the lesions (liver, forestomach, and subcutaneous) produced by 4-dimethylaminobenzene-1-azo-1-naphthalene.

Alkaline phosphatase and glycogen studies on livers of adrenalectomized and desoxycorticosteroneacetate treated rats fed with paradimethylaminobenzene.

Endocrine Pathology Unit studies of the influence of the adrenals and adrenal cortical hormones on the experimental induction of cancer of the liver suggest that desoxycorticosterone has an anticarcinogenic effect as far as the induction of liver tumors with "Butter Yellow" is concerned. Further studies are under way.

Experiments with virgin and pregnant rats of different strains fed with 2-acetylaminofluorene and treated with progesterone were conducted to gain information on (a) the role of pregnancy in the induction of mammary tumors in rats; (b) the combined action of progesterone and the carcinogen; (c) the action of the carcinogen upon the embryos and litters; (d) the role of heredity in the inbred strains on the development of mammary or other tumors induced by the carcinogen.

Investigation of the role of progesterone in the induction of mammary tumors in C3H and A mice indicates that it may play a role in the difference in the incidence of the tumors in these two strains, but with different hormones in the hormonal secretion.

With the production of deciduomas in strains C3H, A, and C57 black ovariectomized mice with different doses of progesterone, it has been demonstrated that there exists a difference in the uterine sensitivity to this hormone in the three strains used. The strain A and C57 black mice need less progesterone than the strain C3H mice to produce large deciduomas.



Results of experiments conducted on the induction of thyroid tumors by thiouracil alone or in combination with carcinogenic agents will help in elucidating the problem of cell susceptibility and the role of epicarcinogenesis and co-carcinogenesis in cancer.

Excellent material for experimental studies on the problem of "Organotropy," especially to the ovaries, has been secured as the result of transplanting an ovarian tumor found in an Osborn Mendel rat. The tumor has been transplanted subcutaneously and intraperitoneally through 22 generations of OM rats and some other strains. The tumor is an adenopapillary carcinoma similar to those described in women. Eighty-four percent of the intraperitoneally implanted females showed a selective implantation, mostly in both ovaries, with formation of big tumors.

Histologic evaluation of transplanted melanoma S-91 and the amelanotic derivatives S-91A in dba and C mice showed many different growth patterns, often in the same section, and other peculiar characteristics. Studies on these tumors are continuing.

Studies of the influence of uterine trauma and of ovariectomy on the five stages of the diestrus cycle in C<sub>3</sub>H mice confirmed earlier reports that it is impossible to produce deciduomas during the normal diestrus cycle in mice. Other findings seemed to indicate that the action of estrogen in mouse cycles is stronger than that of progesterone.

Geographic Pathology Unit studies have been initiated which it is hoped will throw light on the factors responsible for some of the striking differences in the incidence of cancer of certain sites of the body among people in different geographic areas and among different ethnic groups. The first study, which is being carried out in cooperation with hospitals in Israel and New York City, deals with cancer of the genital organs and breast among Jewish and non-Jewish women. The work includes comparative histologic study of neoplastic and preneoplastic tissues from the patients studied, in addition to detailed case histories for the elucidation of the problem of the rarity of cervical cancer in Jewish women.

Gynecologic Pathology Unit. The technique for producing carcinoma of the cervix in mice by means of painting with methylcholanthrene has been applied to a study of the interrelationship of hormones and carcinogens. A study in progress comparing cervical carcinogenesis in castrates and intact females indicates shorter latent period in the castrates.

Cytodiagnostic service rendered the U. S. Public Health Service Dispensary in Washington has resulted in discovery of clinically unsuspected carcinoma in situ in approximately one out of every 150 cases examined.

Studies on the mechanisms of metastasis indicated that interference with blood coagulation by treatment with dicumarol had no influence on the incidence of spontaneous metastasis of a mouse mammary tumor.

A quantitative method has been devised for the introduction of a counted number of tumor emboli into the tail vein of mice.

Short Term Tissue Culture Unit. A tissue culture technique using a sponge matrix gives promise of great usefulness. Small pieces of cellulose sponge, which is not digested by the growing tissues and is not difficult to section after paraffin embedding, are added to the usual nutrient media of tissue culture. After growth of the tissue in culture the sponge fragments can be studied by the same techniques as any tissue. There is preliminary evidence that the cells aggregate to form identifiable tissue units in 1 to 2 weeks. Plans have been made in cooperation with a local cancer clinic to study human cancer by this method. It is hoped that the cellular patterns obtained in this type of tissue culture will contribute information which will be of aid in the classification of tumors. Although the limitations of the method remain to be determined, a wide range of problems could be approached with this tool if a general parallelism should appear between the function and grouping of cells in vivo and the arrangement and function of cell units in the sponge matrix.

Urologic Pathology Unit. The studies of this unit are directed towards elucidating the role of steroid hormones and the endocrine system on the genito-urinary tract, particularly in relation to testicular tumors. The development and application of histochemical and histophysical techniques for use in these studies has received major emphasis.

Studies have been continued on the morphological, histochemical, and histophysical changes occurring during the induction of interstitial cell tumors in the testes of strain C mice by subcutaneous implantation of stilbestrol-cholesterol pellets. Some differences in the time of depletion and/or return to normal were noted in alpha cell populations of the pituitaries of normal, castrated, and estrogen-treated animals. Ultraviolet absorption micro-spectroscopic studies were made of sections of the induced tumors in an effort to determine the absorption peak of the intracellular lipid. The lipid, ceroid, noted in the testes and adrenal glands in estrogen-treated animals was studied by the techniques for phenolase and peroxidase with negative results.

An induced interstitial cell testicular tumor was transplanted in 75 mice divided as follows: 15 males with subcutaneous implants of stilbestrol, 20 castrated males, 20 spayed females, 10 intact males, and 10 intact females, and a spontaneous tumor, thought to be an interstitial cell tumor of the testes, was transplanted to 5 groups of 25 mice each of these same classifications. Results indicated that both tumors had some androgenic activity.

Hematologic Pathology Unit. The primary purpose of the studies in this unit is to accumulate data on the normal conditions of the blood and blood-forming organs in mice and other laboratory animals which will serve as a background for the recognition and understanding of neoplastic and other changes found in various inbred strains of mice. To secure the data on normal conditions, representative animals of both sexes, at two age periods, from six different strains of mice have had complete autopsies with special attention to lymphatic and hematopoietic organs. High and low leukemia strains were included to see whether detectable differences exist in the preleukemic period.

Comparison of the blood of young mice injected with the mammary tumor agent with that from uninjected mice showed no detectable differences so far, but the work is not yet completed.

In an effort to throw further light on the question of whether one of the important factors in leukemia is the failure of the lungs to remove leukocytes, comparative blood counts are being made of blood from the tail, right and left ventricle of normal and leukemic mice.

Other experiments are directed at the production of malignant melanomas and the development of mast cell leukemia. Studies in collaboration with other investigators are being conducted on mammary tumors, the transplantation of lung tissue and development of lung tumors, the effect of a contaminating virus on a line of leukemia, the effect of pyridoxine deficiency on lymphocytes and the development of leukemia, and the effect of gallium on blood and blood-forming organs.

Veterinary Pathology Unit toxicity studies on the effects on normal mice of stable and radioactive gallium and of the peltatins were completed. Further studies will test the effects of these agents on tumor tissues and on other species of animals.

New projects initiated and still under way include work on an autopsy study of all animals which die or are destroyed at the Washington Zoo to determine the incidence and types of cancer which develop in captive wild animals and birds.

Efforts are being made to induce osteogenic sarcoma in dogs by the use of beryllium and to grow transplanted tumors in dogs from fresh dog tumor specimens furnished by local veterinarians. If the latter is successful it will provide suitable material for further research in the nature of tumor growth and possibly also material for use in chemotherapy studies.

Pathology Technology Unit. This unit rendered the following histopathological services to investigators throughout the Institute including the Clinical Research Unit, Baltimore.

Number of blocks cut -----	47,590
Blocks cut serially -----	710
Frozen blocks cut -----	315
Total stained slides -----	67,391
H and E ----	59,508
Special stained slides	7,883
Autopsies -----	15,997
Bottles submitted -----	18,425
Total pieces of tissue cut -----	102,102

LABORATORY OF EXPERIMENTAL ONCOLOGY

San Francisco, Calif.

Michael B. Shimkin, Chief

The Laboratory of Experimental Oncology is located in the Laguna Honda Home, San Francisco, Calif. The work of this laboratory represents a joint undertaking of the National Cancer Institute and the University of California. The staff averages about 50 persons. Both clinical and laboratory studies on neoplastic diseases are conducted, with major emphasis on the former.

With the cancer patient as the focal point of investigation, the work is oriented along four broad approaches: (1) Experimental therapy, providing clinical material for other studies as well as permitting evaluation of such procedures on neoplastic diseases; (2) Physiology, particularly hematologic physiology of patients with neoplastic diseases; (3) Biochemistry, particularly specific biochemical reactions and radioactive tissue studies on cancer patients; and (4) The study of protein fractions of cancer and normal tissues of human and animal origin, utilizing physicochemical and immunochemical techniques.

Among the main achievements of the laboratory last year were:

Discovery of the importance of the lung in the physiologic dynamics of leukocytes in man, and the demonstration of the importance of the leukocyte removal mechanisms in leukemia.

Demonstration by new arteriography methods of the increased vascular supply of tumors in man, and the application of the finding to diagnosis, treatment, and physiologic studies.

Appraisal of several chemotherapeutic agents in cancer in man with negative results, and the study of a patient with melanoma in whom the hypophysis was removed surgically.

Development of accurate quantitative methods for the preparation of stable radio-iodated proteins and their use in laboratory and clinical studies.



Development of a new micro-spectrophotometric method for measuring the state of titration of the acidic and basic groups of nucleic acids.

Space limitations of this report prevent the inclusion of reports on the respective studies conducted at the laboratory. These are given in the laboratory's annual report, copy of which is available in the NIH Library and from the National Cancer Institute.

CLINICAL RESEARCH UNIT  
Ezra M. Greenspan, Acting Head

The work of this unit is carried on at the U. S. Public Health Service Hospital in Baltimore, Md.

Clinical and laboratory investigations of a number of potential or recently developed chemotherapeutic agents were continued. It was shown that alpha-peltatin induced necrosis and temporary shrinkage of lymphoma in man, but that prolonged administration of alpha-peltatin was not feasible because of the development of intractable diarrhea. Studies were undertaken of the possible synergism clinically between this compound and other chemotherapeutic agents.

Study of the influence of prolonged administration of folic acid antagonists on the metabolic and clinical status of patients with solid tumors was continued. It was found that citrovorum factor completely reversed the toxic manifestations as well as the tumor-inhibiting action of a-methopterin and aminopterin in man.

A mucoprotein test was developed which may prove to be an aid in the differential diagnosis of liver diseases in which the symptoms include enlargement of the liver or jaundice.

An heretofore, undescribed anticoagulant property of serum mucoprotein was demonstrated in mucoproteins isolated from the serum of man and other mammals.

Biochemical investigation of a number of metabolic effects of certain chemotherapeutic agents was continued.

A number of laboratory studies were undertaken to determine whether drugs which heretofore have been employed individually in the treatment of tumors may act synergistically.

Study was made of the biological interrelationships of folic acid and citrovorum factor with reference to the antimetabolite, aminopterin.



TUMOR SERVICE - U. S. MARINE HOSPITAL  
Baltimore, Md.  
Emerson Y. Gledhill, Deputy Chief

The tumor clinic, which originally functioned as an individual unit of the hospital, was reorganized during the year. The X-ray department was transferred to the hospital's X-ray department, and the surgical work was transferred to the general surgical service of the hospital. A tumor board composed of representatives from the hospital's medical, surgical, pathologic, and X-ray services was formed.

Statistical record of tumor clinic activities. Fiscal year 1951

	<u>In-patients</u>	<u>Out-patients</u>	<u>Total</u>
New admissions <sup>1</sup>	494	408	802
Readmissions <sup>2</sup>	641	1750	2391
Consultations	196	6	202
Biopsies	181	111	292
Operations - tumor clinic OR <sup>3</sup>	454	193	647
main OR	315		315
Surgical Specimens			1019
X-ray treatments <sup>4</sup> - high voltage	3542	326	3868
low voltage	451	288	739
Radium and radon treatments (Interstitial and surface)	46	15	61
Miscellaneous procedures <sup>5</sup>	12737	358	13095
Follow-up visits (In and Out Patients)	135	1750	1885

1. Transfers from other Marine Hospitals - 119
2. Transfers from other Marine Hospitals - 80
3. Includes biopsies, endoscopic examinations and other minor operations.
4. Figures represent actual treatments and will therefore vary from hospital figures giving number of patients.
5. Requiring the special examining-dressing room facilities.  
Does not include any of the other listed items, but does include other special examinations and dressings not listed above.

EPIDEMIOLOGY SECTION  
A. G. Gilliam, Chief

This section renders consultation services to other units conducting epidemiological investigations and has four major studies under way based on several thousand case histories:

Analysis of certain events in sexual lives of patients with cancer of the breast and cervix in comparison with several other sites.

Analysis of smoking histories of patients with cancer of the lung and larynx in comparison with other sites.

Analysis of occupational histories of patients with cancer of the lung and larynx.

Study of the distribution of mortality from leukemia, by counties, for the United States, 1944-1948.

#### CANCERIGENIC RESEARCH LABORATORY

W. C. Hueper, Chief

The work of this laboratory, located at the Georgetown University Medical School, is along three main lines:

Experimental studies on metal cancerigenesis. A number of metals, metal compounds and minerals with metallic impurities are being studied for cancerigenic effects. Experiments with finely dispersed metallic nickel powder produced a high percentage of malignant conditions in the animals used. Uranium injected intrafemorally and intrapleurally also produced malignant neoplasms. Tests on arsenic, chromium, chromite, asbestos and beryllium are still under way.

Experimental investigations on shale oil and oils produced from coal. A low percentage of skin tumors developed in animals painted with crude shale oil and crude synthetic oil produced by the Bergius process from coal by the Bureau of Mines. Oil produced by the Fischer-Tropsch process gave negative results so far.

Experiments with a new weed killer (Isopropyl-N-Phenylcarbamate) for potential cancerigenic properties gave negative results.

#### EDITORIAL OFFICE

Ross C. MacCardle, Acting Scientific Editor

Edith E. Parris, Managing Editor

The editorial office reviews all research manuscripts written by members of the scientific staff of the Institute and all scientific manuscripts submitted by outside authors for publication in the Journal of the National Cancer Institute. It conducts all editorial and administrative work incident to the publication of the Journal of the National Cancer Institute and reprints therefrom, and maintains a reading room of current scientific journals.

Work was begun on an index for the first 10 volumes of the Journal, and on a study of scientific nomenclature in the interest of establishing the best standards of terminology in the Journal.

CANCER CONTROL BRANCH  
R. F. Kaiser, Chief

During fiscal year 1951, the Cancer Control Branch had as one of its major objectives the reduction of mortality due to breast cancer. A concerted effort was made to carry out a program which would accomplish such a reduction, centering around the development, production, and utilization of two motion picture films on breast cancer. One film was designed specifically for the medical profession and stressed diagnostic procedures in relation to breast cancer. The other was a lay educational film entitled "Breast Self-Examination." This film demonstrated a technique of monthly self-examination for women, urged them to carry out the examination procedure and, in the event breast masses were discovered, to see their physicians for further instruction. Through the combined efforts of the Cancer Control Branch and the American Cancer Society, approximately one million women have seen this film since its release. To date, print sales of the film have reached a total of approximately 1,000 copies, a figure which represents four or five times the number of sales of any other public health educational film.

A project to evaluate the effectiveness of this film was undertaken through the joint sponsorship of the Iowa State Department of Health, the Iowa State Medical Society, and the Iowa Division of the American Cancer Society. Efforts to improve and expand cancer diagnostic services and facilities have continued. This has been effected partially through the State grant-in-aid program and has resulted in the establishment of a sizeable number of cancer diagnostic clinics. Also the Branch has sponsored training of 3 full-time and 2 half-time clinical trainees during the year. It is anticipated that these doctors, upon completion of their training in the diagnosis and treatment of cancer, will form the nuclei around whom additional cancer diagnostic clinics will be established.

The diagnostic acumen of undergraduate medical students has been sharpened through the cancer teaching program which reached all but one of the Nation's medical schools during the year. In cooperation with the American Cancer Society, the Branch developed and produced a diagnostic film entitled, "Gastrointestinal Cancer: The Problem of Early Diagnosis," which is designed to present diagnostic procedures in this field to both undergraduate and graduate physicians.

The major portion of production work was completed on the uterine cancer diagnostic film. A shortened version of the film, "Challenge: Science Against Cancer" produced jointly with the National Cancer Institute of Canada, and entitled, "The Fight: Science Against Cancer," was one of the finals in the competition for the award for the best documentary film given by the Academy of Motion Picture Arts and Sciences in Hollywood.

Another objective of the Branch has been the bringing of other professional groups into active participation in the cancer program. Undergraduate

cancer teaching grants were made to all but two of the approved dental schools and, for the first time, grants were awarded to the approved colleges of osteopathy.

In an effort to extend the available knowledge of cancer to the nursing profession, the Branch, through its Nursing Section, has entered into an educational program with Schools of Nursing, universities, and other educational institutions to make adequate cancer knowledge available to nurses and to improve nursing care to cancer patients.

A public health educator was added to the staff to provide consultation service to State and local health departments and nonofficial agencies, to assist in coordinating cancer educational activities with the general health educational program and to assist in the evaluation of cancer educational materials and techniques.

Through the mechanism of special project grants, working arrangements have been entered into with a large number of organizations outside of State health departments. Twenty-six new grants were recommended by the National Advisory Cancer Council for cancer control special projects during the year, along with continuation of 38 already established projects. A large number of studies related to preinvasive cancer or carcinoma-in-situ were supported. Extensive efforts were made to investigate environmental factors as related to the causation of cancer. Of significance in this area was the discovery of the relationship of finely dispersed metallic nickel powder to the causation of cancer in rats.

In an effort to determine the extent of the cancer problem, the results of the morbidity surveys conducted under the direction of the Cancer Control Branch in previous years were analyzed, and reports on Atlanta and San Francisco were published.

The search for new diagnostic tools has continued. An evaluation of many of the diagnostic tests reported in the literature has been accomplished during the year and the program now has reached the stage of development of diagnostic tests. The first cancer Diagnostic Test Conference, organized and supported by the Branch, was held in Oct. 1950. Appreciable gains have been made in this area, although there is not a simple diagnostic test procedure available for the discovery of all cancer. Further investigation has been carried out and supported in relation to the applicability of the cytologic test for cancer.

The radium loan program has continued, with 53 institutions now participating.

Further details of the work of the branch are included in the following reports from Section Chiefs.



FIELD INVESTIGATION SECTION  
J. E. Dunn, Chief

The interests of the Field Investigation Section continued to be primarily in the field of the development and evaluation of cancer diagnostic tests, and the study of the cytologic technique for the diagnosis of cervical cancer.

The Clinical Trials Unit established in 1948 in the Department of Pathology, University of Washington Medical School, has continued its work on development and evaluation of cancer diagnostic tests. Work has been completed on the evaluation of a number of proposed tests and a number of papers have been published or are in various stages of manuscript preparation on the Huggins' iodoacetate and least coagulable protein tests; the Black methylene blue and heat coagulation tests; the measurement of serum trypsin and chymotrypsin inhibitors as cancer diagnostic tests; the test based on the serum-vanadate reaction; serum musoprotein measurement as a cancer diagnostic test; and the use of a combination of tests for cancer diagnosis. It may be said that none of these individual tests is sufficiently sensitive and specific to be used for a cancer screening test. Because of the high correlation of most of these test procedures through their dependence on the changes in major components of the blood proteins in cancer patients, the advantages obtained in combining these tests are not great. This still remains as a possibility, however, for tests that are independent.

Work has continued on the development of the complement fixation test for cancer using the Brown-Pearce tumor in the rabbit. The results of this test so far indicate that it has a high degree of specificity and an encouraging degree of sensitivity.

Considerable work is being done in an attempt to purify the antigenic principle of the Brown-Pearce tumor. Techniques being used include alcohol fractionation, chromatography, fractionation with the Kirkwood Electrophoresis Convection cell, ultrasonation of crude tissue suspensions, and others. The observations on uses of the test are also being extended to other species, particularly the mouse, using transplantable tumors and strains with a high incidence of spontaneous tumors.

The Cancer Investigation Center at Hot Springs, Arkansas, continued the cytologic screening of the admissions to the Medical Center during the first half of the fiscal year. This unit has now been moved to the University of Tennessee where it will be affiliated with the Department of Pathology. The work to be carried on here will be directed toward determining the usefulness of cytology as a cancer case-finding procedure. Since vaginal cytology for female genital tract cancer, particularly cervical cancer, seems at present to offer the greatest promise for such use of the procedure, this will be given major emphasis. The questions that must be answered before the case-finding potentialities of the procedure can be realized are as follows: (1) Does the

preinvasive lesion designated as carcinoma-in-situ almost invariably progress to invasive cancer; (2) does all invasive cancer of the cervix begin as carcinoma-in-situ; (3) and what is the duration of carcinoma-in-situ. These are the questions that this Unit will attempt to answer. Plans are being made to carry out a cytologic screening of the general female population of Memphis to obtain experience with such an application of this procedure and through this and successive screenings of the same population to obtain prevalence and incidence rates that will provide direct and indirect answers to the questions stated above.

A study designed to measure objectively the performance of cytology technicians was carried out, the results analyzed, and may eventually be published. A group of patients with suspicious cytologic findings, but in which the diagnoses had not been confirmed by cervical biopsy, were recalled for further vaginal cytology and multiple cervical biopsies.

For the assistance of those engaged in the evaluation of cancer diagnostic tests a manuscript was prepared and published, setting down the criteria for such evaluation. A statistical procedure was proposed for the analysis of experimental results for the rejection or tentative acceptance of a cancer diagnostic test. Considerable work has been done in the statistical analysis of such results from the Clinical Trials Unit and the four collaborating university groups.

STATE AID SECTION  
Noka B. Hon, Chief

The State Aid Section has the responsibility for coordinating cancer control activities of the Cancer Control Branch with the State health departments through the various regional offices.

The major emphasis has been in three areas: (1) The review and analysis of State cancer control programs financed by grant-in-aid funds; (2) Furnishing of consultation services to State and local health departments, regional offices and nonofficial participating in the control program; (3) Assisting in coordinating official and nonofficial cancer control activities in many of the States.

Many of these activities are carried out cooperatively with other sections of the Cancer Control Branch. The Cancerigenic Studies Section has cooperated in stimulating State health departments to conduct studies themselves or assist in studies to define the scope of occupational cancer hazards in relation to occupational groups and to cancer in general.

The Nursing Section has cooperated by providing guidance in the nursing phases of the State cancer control programs, directing nursing studies and furnishing personnel to participate in or conduct studies.

The Biometrics Section has provided personnel for consultation services to State health departments and other agencies in nine States and has reviewed the cancer register programs in six States. This Section has also cooperated in work done with the American College of Surgeons to develop a formula for determining the number of cancer diagnostic and treatment facilities needed in State control programs; and in preparing a revised manual of instructions for operating a cancer register in a local health department. This revision is based on experience gained in a 3-year pilot cancer morbidity reporting and register program conducted by the Maryland State Department of Health in cooperation with the NCI.

In collaboration with the Field Investigation Section, plans have been made for a study in one county of Georgia to help in determining the practicality of the cytology test as a case-finding technique in cervical cancer. The State health department, local physicians, and a competent cytologist supported by a special project grant, will participate. Cytology tests will be repeated annually on the women participating in the study. The data gathered will provide valuable prevalence and incidence data not now available, as well as experience in the case-finding potentialities of this procedure. Reliable techniques for finding early cancer are greatly needed.

CANCERIGENIC STUDIES SECTION  
W. C. Hueper, Chief

The Cancerigenic Studies Section is concerned with the relation of environmental factors to cancer. The activities include the planning and collaboration in occupational cancer surveys, consultation services, and laboratory research. Work in the latter category is described under the heading "Cancerigenic Research Laboratory" in the report of the Research Branch of the NCI. A brief summary is given below of the status of the several surveys planned by this office and aided by special cancer control projects grants.

An occupational cancer survey undertaken in Ohio 3 years ago was completed. This included:

A study of the disease situation among past and present employees in the chromate plant of the Diamond Alkali Company, Painesville.

A statistical study of all cancer deaths in the male population of Ohio from 1948 to 1950.

A study of deaths from cancer among rubber workers in the Akron area. As this showed a high incidence of deaths from brain tumor, it suggested the existence of an exogenous agent responsible for cancers of the central nervous system. As a result of this finding, the State health department has been given a Special Control Grant to support an epidemiological study of cancers of all types among rubber workers in Ohio.



In Connecticut, a study is being made by the Cancer Control Division of the State department of health. An analysis of the cancer deaths according to regions showed a statistically significant high total cancer mortality among the population of the Naugatuck Valley region in which rubber and chemical plants as well as nonferrous metal factories are located. The statistics will be analyzed further.

In New York some 3,000 patient records at the Roswell Park Memorial Hospital, Buffalo, are being studied. There are no significant findings as yet.

In New Jersey the occupational cancer study under the State department of health is in its third year. A considerable amount of well-controlled and reliable data on approximately 2,000 deaths of males from cancers affecting the lung, larynx, nasal, sinuses, bones, bladder, and hematopoietic system has been collected from death certificates. Study of the data in relation to industrial areas of the State is continuing.

The study in Pennsylvania, which is under the direction of the Division of Industrial Hygiene, State Department of Health, is concerned with occupational relations to cancer of the lung, as revealed by death certificates for the years 1947 to 1950. Progress on this study has been slow.

Two studies are under way in Colorado---

A study in cooperation with the Department of Industrial Medicine, University of Colorado Medical School. An analysis of the death certificates for a 2-year period shows that there is a definite difference in cancer incidence in different parts of the State. The reasons for these variations are being studied. Cancer morbidity data secured from the State Cancer Control Division's Cancer Registry are also being studied for relation to occupational factors. Special attention in this survey is given to the possible influence which contact with radioactive ores in uranium mines and mills, with native asphalt, crude and processed oil, pesticides, solar irradiation, chemicals used in rubber goods and tar, soot and mineral oil employed or generated in steel plants may have on the incidence, occurrence, and topographical distribution of cancers among the population of Colorado.

A survey of radioactivity in the uranium mines and mills on the Colorado Plateau planned in cooperation with the Industrial Hygiene Division, USPHS and the Colorado State Department of Health. These investigations have shown that the radioactivity in the mines and in some operations at the mills entail radioactive hazards which are equal or even higher than those sustained by workers employed in the radioactive mines of Schneeberg and Joachimsthal. As the result of these observations, extensive changes in the production and processing procedures have been instituted in several mills and in many mines where the radioactivity was found to be high.



In California a lung cancer morbidity survey, conducted by the State department of health, indicates that mine and smelter workers of nonferrous metals who come in contact with arsenic dust and fumes represent a definitely higher proportion of lung cancer patients than miners of coal mines or iron mines. There is also a higher incidence than normal of lung cancer among oil refinery workers and a positive statistical correlation between cigarette smoking and lung cancer.

In an effort to create additional research facilities and provide competent training in epidemiologic and experimental methods of investigation in the field of environmental cancer, plans are under way to establish environmental research units in universities. Two have already been started and negotiations for others are under way.

PUBLIC HEALTH NURSING SECTION  
Rosalie I. Peterson, Chief

The work of the nursing section is designed to help nurses acquire adequate cancer knowledge, improve nursing care to cancer patients, and increase the nurse's effectiveness in cancer control activities. The program includes direct services to official state health agencies and universities, cooperation with nursing groups in the improvement of cancer teaching in nursing schools, and the development of new educational tools.

Direct Services in the year under review include:

A 1-week conference on the "Integration of Cancer in the Basic Nursing Curriculum," held in Nebraska. Thirteen schools of nursing as well as a number of other agencies participated.

A 2-week conference on cancer nursing held at the University of Kansas Medical Center. Members of nursing school staffs and personnel from State and local health departments participated. The work was designed to improve cancer nursing in the basic curriculum.

Seven 1-day and seven 2-day Nursing Institutes in the States and Puerto Rico.

A two-credit course (60 hours) in cancer nursing at the University of Hawaii.

A 3-day conference to evaluate the results of a 3-week cancer nursing course conducted by the Section the previous year at the University of Minnesota for representatives of 30 universities. (Twenty persons who participated in the first conference came to Washington at their own or school expense to attend this evaluation conference.)

In addition numerous talks have been given at meetings of nurses' organizations and cancer societies, and advisory service given to regional offices and State health departments in connection with various cancer nursing projects conducted in their areas.

A monograph on "Cancer Nursing in the Basic Professional Nursing Curriculum," prepared by a committee sponsored by the Nursing Section, a major contribution in the field of cancer nursing education, is in press.

Other educational activities included the development of a 100-item opinionnaire to be used in ascertaining the attitude of student nurses toward cancer, as a guide in improving these attitudes, and in determining areas in which student nurses need more information; the writing of a series of articles on cancer or cancer nursing for publication in professional nursing journals; the preparation and distribution to appropriate agencies and institutes of various kinds of educational reference materials.

TRAINING AND PROJECT GRANTS SECTION  
R. F. Kaiser, Acting Chief

Cancer teaching grants recommended by the NACC for 79 medical schools amounted to \$1,847,005; for 39 dental schools, \$182,190; and for 6 schools of osteopathy, \$138,190.

Cancer control special project grants amounting to \$986,123 were recommended to continue 38 already established projects and to initiate 26 new ones.

PROFESSIONAL TRAINING PROGRAM  
R. R. Spencer, Head

There were 111 full-time and 2 half-time trainees on duty on July 1, 1950. Forty-eight were specializing in surgery, 29 in radiology, 24 in pathology, 7 in internal medicine, and 5 were training in more than one specialty.

Approximately 90 applications for traineeships beginning July 1, 1951 were received; 56 appointments effective as of that date were made.

GRANTS AND FELLOWSHIPS BRANCH  
Ralph G. Meader, Chief

The Grants and Fellowships Branch carries on the following activities:

Administers funds appropriated for:

The support of basic and applied research in cancer and closely related fields carried on in non-Federal institutions.

The granting of fellowships to qualified investigators or students for training and special research in these fields

The construction of facilities in non-Federal institutions for the housing of research and training activities in cancer.

Provides the Surgeon General, the National Advisory Cancer Council, and other reviewing panels with adequate information to reach decisions as to allocation of funds designated for the above purposes.

Stimulates and promotes cancer research and the diffusion of knowledge thereon.

Administration of grant and fellowship funds.- In discharging this function the Branch is assisted by the NIH, Division of Research grants, the Budget and Fiscal Section of which handles routine audit and fiscal control of allocated cancer grant funds, subject to the direction of the Branch Chief.

Research grants. Three hundred and fifty-five grants amounting to \$3,859,728 were recommended by the Council and approved by the Surgeon General.

Construction grants. No new funds were appropriated for construction grants this year. Payments of \$5,280,777 were made from funds previously authorized. As of June 30, 1951, 24 construction projects were completed, 22 underway and 9 were in the initial stages.

Research fellowships. Of the 364 applications received, 293 were passed by the Central Qualifications Board, and 173 of these were recommended by the Cancer Speciality Board. Twenty-four were for citizens of foreign countries to study in the US and 3 were for Americans to study abroad. There were 8 pre-doctoral, (bachelor), 71 predoctoral, (masters) 86 postdoctoral, and 8 special fellowships.

Provision of information to the surgeon. General and advisory groups.- In preparing grant applications for consideration by the NACC, the Branch is assisted by the Project Review Section and the Study Sections of the NIH Division of Research Grants. The diversity of approaches to the cancer problem is illustrated by the fact that the services of approximately 15 study sections are used in reviewing the applications. Experts in appropriate fields cooperate with the branch in reviewing applications for which there is no study section. The Gastric Cancer Committee reviews all applications dealing with gastric cancer.

An analysis of grants awarded in the fiscal year 1949-50 showed that 38 percent were for studies in the field of carcinogenesis (external stimuli, 15 percent; internal stimuli, 12 percent; mechanisms, 11 percent); 30 percent were in the field of tumor development (tissue synthesis, 20 percent; host-tumor relations, 10 percent); 25 percent were in the clinical field (epidemiology,

1 percent; diagnosis, 8 percent; therapy, 16 percent); 2 percent were grants to assist in the publication of special scientific reports, proceedings of conferences, etc., and 3 percent were for study of fundamental biological problems rather than for any particular cancer problem. The remaining 2 percent of the grantees had not yet returned their questionnaires.

Stimulation and promotion of cancer research and diffusion of knowledge.- The Branch works with two special committees which encourage and advise on research in their respective fields, gastric cancer, and radiation studies. At the last meeting of the Gastric Cancer Committee it was recommended that another national conference on Gastric Cancer be held in the near future. The Radiation Committee has made recommendations concerning types of studies to be undertaken in that field.

The Branch provides consultation services on request to individuals to aid them in planning research programs, securing personnel and equipment, construction of laboratory facilities, etc. Investigators with common interests are brought together to help avoid unnecessary duplication of effort. Upon request, advice is given qualified investigators as to problems or areas in cancer research where new or additional work is needed.



NATIONAL HEART INSTITUTE

C. J. Van Slyke, Director

C O N T E N T S	Page
Introduction.....	47
Advances in heart research.....	47
Grants for heart disease research.....	49
Research on blood.....	50
Intramural research.....	50
Section on Chemical Pharmacology.....	50
Section on Cellular Physiology.....	56
Section on Chemistry of Natural Products.....	59
Section on Metabolism.....	65
Section on Cardiovascular Hemodynamics.....	68
Section on Kidney and Electrolyte Metabolism.....	69
Section on Technical Development.....	74
Section on Gerontology.....	77
Section on General Medicine and Experimental Therapeutics.....	80
Public Health Methodology.....	83
Grants and training.....	88
Research Grants Program.....	90
Status of Heart Research Grants, June 1, 1951.....	93
Distribution of Current Research Grants by Age of Principal Investigator...	94
Construction Grants.....	95
Grants for Improvement of Undergraduate Medical Education.....	95
Research Fellowship Grants.....	96
Heart Traineeships.....	96
Technical Services.....	97
Framingham Heart Disease Epidemiology Study.....	97
Biometrics Research Section.....	98
Heart Information Center.....	99



## Introduction

Advances on many fronts marked the third year of the National Heart Institute, established in 1948 to lead and coordinate the Public Health Service program against heart disease. In intramural research, considerable progress was made toward increasing basic and clinical knowledge of the underlying disease processes affecting the heart and circulation. Through a comprehensive grants program, expanding research activities in universities and hospitals throughout the nation were fostered and supported. Significant gains were also made in training in both the research and clinical aspects of the heart diseases. Through cooperative efforts with the Bureau of State Services, the public health control of heart disease was stimulated and moved steadily forward in many States and local communities.

The impact of heart disease on national health and well-being is great. It accounts for 1 out of every 2 deaths. Nearly 10 million Americans have heart disease. The suffering, disability, and economic loss resulting from it are incalculable. The eventual solution of the heart disease problem depends largely upon the discovery of new knowledge concerning its causes, prevention, treatment, and cure. Hence the principal emphasis of the National Heart Institute is on research.

## Advances in Heart research

The cause of arteriosclerosis and coronary artery disease is a subject of controversy. Various investigators hold that the fundamental metabolic defect which results in the sclerotic or hardening process in arteries is one that precludes normal handling of cholesterol, fosters the formation of large protein-fat complexes in blood plasma, or impairs the ability to regulate phospholipid metabolism. Institute investigators premise that the three possibilities are interrelated one with another, with metabolic handling of small energy-yielding particles, and with the synthesis of abnormal protein molecules. Their approach has developed chemical methods for the estimation of serine, ethanolamine, and choline which are the basic building blocks of the fatty substances in the body called phospholipids. The methods will permit, for the first time, a definitive study of the individual components of the four distinct types of phospholipids.

Initial studies have been completed on the metabolic transformation of cholesterol through the use of a number of enzyme systems and have isolated fragments of the molecule. Continuation of these investigations will make available information on the critical points of the molecule which are presumably responsible for its vulnerability to metabolic transformation and on potential precursors of cholesterol. Other studies on the mechanisms involved

in the biosynthesis of protein have clarified certain generalities of the process in relatively simple biological systems. Biophysical techniques have been expanded and now permit the isolation and characterization of proteins and protein lipid complexes.

While the major cause of hypertension is not known, the simple lowering of blood pressure is conducive to lesser cardiac disability, consequently to a longer and more useful life. Considerable progress has been made by the Institute's researches in exploration of the endocrinological aspects of the hypertensive condition, the study of synthetic blood pressure lowering agents, and the isolation from natural sources, both plant and animal, of pure principles which have the property of raising or lowering blood pressure. The serial study of a number of drugs which have the ability to lower blood pressure has determined certain of their inherent limitations. This has led to establishment of experimental hypotheses that permit the synthesis of other chemical agents which will have more suitable biological properties.

Investigations in the broad problem of the failing heart seek to clarify biochemical reactions in cardiac muscle whereby the energy required for contraction is provided from chemical nutrients which serve as fuel. The underlying biological mechanisms responsible for raising small carbon fragments from low to high energy values have been isolated for study. Certain of the mechanisms through which high energy carbon fragments are utilized in transfer of energy from one organic complex to the other have been clarified. Progress has been made in isolation and study of tissue catalysts involved.

Other studies have provided data which are applicable to better understanding of the circulatory system as a whole, the control of fluid and electrolyte composition of the body, and mechanisms implicated in the syndrome of heart failure. Certain kidney mechanisms concerned with retention or excretion of salt and others which determine acidification or alkalinization of body fluids have been isolated. These accomplishments are essential to development of a more rational basis for the use of diuretics. Experimental preparations have been produced which simulate the circulatory phenomena in cardiac failure. It is quite certain from these studies that the endocrines play a role which is as important as either the heart or the kidney in the precipitation and continuation of heart failure.

The cardiac patient is vulnerable to surgical procedures involving strong sedation and general anesthesia, particularly if the latter is required for more than a short period of time. Study of pentothal, the most commonly used "short-acting" intravenous anesthetic, has found that the drug has serious disadvantages of progressive localization in the fat in the body and a slow rate of inactivation. Administration for a long period of anesthesia results in accumulation of a large amount in the body, which produces a post-anesthetic depression of excessive duration. This led to the study of a number of new compounds derived or related to barbituric acid, and one that appears to have suitable characteristics is under clinical trial.



The most commonly used anti-clotting drugs produce their effect through an indirect action on the clotting mechanism and are now known, as a result of Institute investigations, to be inherently difficult to control. On the other hand, heparin and heparin derivatives, which are more suitable for such a purpose, are biological in origin, short on supply, and inordinately expensive for continued therapy. Work has been undertaken in the expectation of devising a suitable synthetic substitute for heparin which can be produced at low cost.

Research on instrumentation has also been rewarding. Advances include the development of a mechanical pump which permits, in the experimental animal, the complete bypassing of blood from either of the two sides of the heart. The advantages which accrue to cardiac surgery from the availability of a bloodless interior chamber of the heart are under study. The device is not yet ready for application to the human. Plastic perfusion pumps and apparatus have been designed and are in use in study of the function of completely isolated organs. Preliminary work has been completed on a new optical tool to analyze the fluorescent light emitted by low concentrations of organic substances including drugs. When perfected, it will constitute a powerful tool in the exploration of both new and old therapeutic agents.

#### Grants for heart disease research

Important discoveries were made by a number of scientists whose research was supported by National Heart Institute grants. Studies were conducted on all aspects of heart disease, with many investigations dealing with the major problems of rheumatic fever, hardening of the arteries and high blood pressure.

A valuable contribution to the treatment of coronary thrombosis was made with the discovery of a method to diminish and, in many cases, eliminate the crushing pain which accompanies a heart attack. This research found that an ethyl chloride spray, applied externally, will "freeze" the pain centers and stop the spasms which cause contraction and impairment of the surrounding circulation. Once the immediate pain has been relieved, it apparently does not recur and thus allows the patient to conserve strength for the healing period.

Among the achievements accomplished through surgical research was development of a new technique for the repair of mitral insufficiency, the leaky valve condition that often occurs in rheumatic heart disease. In this method a piece of the pericardium, the sac enclosing the heart, is pulled through the heart and attached so that it will loosely move against the damaged mitral valve. The operation, performed under control of the surgeon's finger within the heart, has been immediately effective in a high percentage of cases treated.

Studies in hypertension have revealed that nearly all persons with high blood pressure have a chemical in their blood which is not present in individuals with normal pressure. This powerful substance, called pherentasin, is

found in very minute amounts. When injected in rats, it quickly raises blood pressure. The discovery points the way to a possible new treatment for hypertension. The scientists are now studying drugs which may neutralize pherentasin and thus help to keep blood pressure normal.

### Research on blood

Of increasing importance in view of the international situation, the action in Korea, and the threat of atomic bombing is the National Blood Program. Responsibility for research and development of blood and its derivatives was delegated to the National Heart Institute. More than a score of expert investigators in this field were given grants to carry out research leading to stockpiling, more economic use of blood through use of derivatives, longer periods of storage and better methods of preservation, as well as new uses of blood and its component parts.

Remarkable progress has been made. Resuspended red blood cells can now be stored up to 3 months. Globin derived from red cells which are discarded in present methods has been found to be effective and safe in treating shock when given with salt or glucose solution. This globin might substitute for plasma and reduce the amount of plasma needed. A new method for separating stable fractions of plasma from those fractions which are easily destroyed by standing or by changes in temperature was developed. Automatic devices were designed to make sure that plasma sterilizing machines function properly. Advances were also made on processes for the partial recovery of out-dated blood.

### INTRAMURAL RESEARCH

James A. Shannon, Associate Director (In Charge of Research)

#### SECTION ON CHEMICAL PHARMACOLOGY

Chemical methods for the estimation of therapeutic agents and other organic compounds in biological material have been developed for the following compounds:

Drugs and drug metabolites: Theobromine, various methyluric acids, ephedrine, various barbiturates, and various Dibenamine-type adrenergic blocking agents.

Normally occurring biological materials: Ethanolamine and serine using the dinitrofluorobenzene reaction. The methods were applied to the determination of ethanolamine and serine in phospholipids. The reaction has also been used to estimate phenylalanine indirectly, following its microbiological decarboxylation to phenylethylamine.

Catechol amines such as adrenalin, noradrenalin, dihydroxyphenylalamine and hydroxytyramine using fluorescent paper chromatographic technique.

Tyrosine by a new colorimetric procedure.

The physiological disposition and fate of drugs in the body.-

#### ANTICOAGULANTS

Tromexan: Studies on Tromexan, a dicumarol analogue said to be safer than dicumarol, are almost complete.

A metabolic transformation product has been isolated in pure form and tentatively identified as a derivative of Tromexan with an additional OH group meta to the lactone ring in one of the benzene rings. The anticoagulant activity of this compound is under study.

Tromexan retains most of the undesirable characteristics of dicumarol. Although it is absorbed more rapidly, its onset of action is not increased to an important degree. Its main advantage, its rapid rate of metabolism, permits overdosage to be more easily rectified. However, clinical experience with the drug has proved disappointing. In many subjects, it is difficult to maintain a constant therapeutic prothrombin response even with numerous prothrombin time determinations. Subjects change their sensitivity to the drug without warning.

Dicumarol: A method of administering dicumarol that minimizes hyperthrombinemia and bleeding is under study. By experiments which relate plasma levels of the drug to prothrombin response it has been demonstrated that the administration of relatively large, infrequent doses of Dicumarol gives better control of therapy than the administration of daily doses, a technique currently in wide use.

#### BARBITURATES

Pentothal: Tolerance to the effects of pentothal has been demonstrated to occur rapidly during the time of a single injection. This tolerance disappears as the drug is metabolized. The phenomenon cannot be explained on the basis of an increased transformation rate of pentothal; it is a true adaptation of the tissues to the depressive effects of the drug.

Nembutal: Nembutal, the oxygen analogue of pentothal, is longer acting than pentothal because it is only slightly localized in fat. This constitutes another example of how the activity of a therapeutic agent may be drastically affected by the extent of its tissue localization. Although, in the dog, nembutal metabolizes more rapidly than pentothal, the reverse is true in man. Species reversals of this kind are important considerations in the overall interpretation of toxicity and other pharmacological data.



Kemithal: Preliminary results with this drug, a sulfur containing British importation containing an allyl and a cyclopentenyl side chain, indicate that this compound is also ultra-short acting because it localizes in fat and not because it undergoes a rapid rate of metabolic transformation.

#### Xanthine Diuretics (caffeine, theophylline, and theobromine)

Methods were devised for the estimation of the three compounds in biological material. The physiological dispositions of theophylline and caffeine have been studied. The main points of interest are their distribution -- they are distributed almost evenly throughout total body water -- and their rates of metabolic transformation which are relatively slow, 10-15% per hour. However, even heavy coffee drinkers usually start the new day with minimal amounts of caffeine in their body.

The major transformation product of theophylline has been demonstrated to be 1:3 dimethyluric acid. This finding solves a long-standing dispute as to the fate of a methyl xanthine in the body. The oxidation of theophylline, unlike xanthine, is not catalyzed by xanthine oxidase. This raises the question whether an alternative mechanism for the oxidation of xanthine is present in the body.

#### Adrenergic Blocking Agents

After the i.v. administration of Dibenamine, part of the drug is rapidly destroyed and part is localized in fat from which it is slowly released over a period of days. A close correlation between Dibenamine fat levels and the degree of adrenergic blockade has been demonstrated.

Chemical evidence has been obtained which indicates that Dibenamine does not act in the body through the formation of a cycloimmonium structure.

Phenoxyisopropyl N-benzyl-N-betachlorethylamine (SKF 688) is a dibenamine derivative which in large doses, orally, can produce blockade. The compound is undergoing extensive clinical trial. It also localizes in fat but to a considerably greater degree than dibenamine. It is presumably more effective for this reason.

#### Analgesics

The fate of demarol in man has been studied. 10 to 15 per cent is excreted unchanged, and about 30-40 per cent is excreted as de-esterified demarol.

Demarol is surprisingly stable in man, metabolizing only 10-15 per cent per hour. The drug accumulates in the body when given in the usual therapeutic dosage. These results suggest that stability may not be an important factor in the duration of action of Demarol and also suggest that tolerance may set in more rapidly than has usually been considered to be the case.



Dogs and rats metabolize demarol extremely rapidly. This may explain why these animals can tolerate much larger doses of Demarol than man, and why the phenomenon on tolerance is difficult to demonstrate with these animals.

The administration of demarol to women in labor, and comparison of plasma drug levels in mother and newborn, show there is no barrier to passage of drug into the foetus.

#### Development of new compounds for pharmacological and clinical studies

##### Compounds for treatment of cardiac arrhythmias:

A large number of procaine analogues have been screened in dogs for their antiarrhythmic activity. Several compounds, especially those containing an ether linkage between the aromatic ring and the diethylaminoethane moiety show considerable activity, but none are as nontoxic and effective as procaine amide.

Procaine amide has been in the hands of physicians for the past year under the trade name of Pronestyl. It has achieved considerable success in the suppression and control of ventricular extrasystoles, in the suppression of attacks of ventricular tachycardia even in quinidine resistant cases, and in the suppression of arrhythmias occurring during anesthesia. The drug is showing some promise as a prophylactic to be given before operations involving chest surgery.

##### Short-acting barbiturates for anesthesia:

Pentothal is too stable in the body to be short-acting after sustained administration. In the search for an unstable barbiturate, three compounds, the oxygen, sulfur and N-methyl analogues of allyl, bromcyclohexenyl barbituric acid were shown to metabolize rapidly in dogs. These compounds have been tested in man. The N-methyl derivative was found to be toxic. The oxygen analogue was too stable in man. However, the sulfur analogue (DP 2365), in six experiments, has shown promise as an anesthetic. For comparable depth of anesthesia, subjects awake sooner and with considerably less post-anesthetic somnolence than after Pentothal. Surprisingly, in the few subjects studied, the compound exerts considerably less depression on respiration than does Pentothal. Further work on this compound awaits the availability of more drug.

#### The biological significance of choline and other nitrogen-containing alcohols in biological fluids and tissues

Choline: Unexpected difficulties arose in the application of our choline method to plasma. Previous data had indicated that the method for free choline was specific. Reinvestigation showed that in the counter-current technique, some choline-containing material hydrolyzed to choline. An additional step in the method appears to remove this interference. Another

difficulty involved impurities in the ethylene dichloride solvent which decomposed the choline-iodide complex. The impurities have been traced and steps taken to prevent their recurrence. The sensitivity of the method has been considerably improved.

A method for the determination of phosphorylcholine has been developed.

Ethanolamine and Serine: Using a new reaction involving the use of dinitrofluorobenzene, methods have been evolved for the estimation of ethanolamine and serine. These methods have been applied toward a study of the cephalin type phospholipids in whole blood and plasma. The countercurrent distribution technique has been used to indicate the identity of the measured substances. Preliminary results demonstrate a considerable amount of serine and ethanolamine-containing phospholipids in red cells. On the other hand, the amount of these substances in plasma is small. The phospholipids in blood can be accounted for in terms of choline, serine and ethanolamine. There is no indication of the presence of phospholipids of an unknown type in whole blood.

#### The use of isotope-labeled drugs in metabolism studies

The ultimate fate of barbiturates in the body is under investigation, particular interest centering in the oxidative degradation of the alkyl side chain, a route of degradation which, although common to a variety of drugs, is not well understood. In order to avoid making assumptions concerning the structure of metabolites, a limitation in the use of labeled material as used so far, radioactively labeled drugs will be administered, and all radioactive substances appearing in the excreta will be completely fractionated. For structure determination, individual active fractions will be added as tracers to large pools of excreta from animals receiving unlabeled drug. Re-isolation of the fraction using the tracer as a guide should provide sufficient material for characterization. Addition of tracer metabolites from small animals to pooled material from human sources should also permit the study of barbiturate metabolism without the necessity of administering radioactivity to humans.

Synthetic procedures for the preparation of Pentobarbital and its sulfur containing analogue, Pentothal, labeled with carbon<sup>14</sup> in positions 2 or 5 have been worked out.

#### A study of factors influencing drug activity

A variety of physiological factors may affect either the route of metabolism or the activity of a drug. As the first in a projected series of investigations of these factors, the effect of sex hormones on barbiturate metabolism is being studied. Previous work, confirmed in these laboratories, has shown that for those barbiturates with longer alkyl substituents at position 5, the duration

of anesthesia in female rats is generally twice that in males after a standard dose. Castration of the males lengthens sleeping time to that of females, but the original sex difference in response can be restored by administration of testosterone to the castrates.

Since there is no significant difference in the blood levels of barbiturate at which female rats, normal males, castrates, or castrates receiving testosterone awake, it may be that the hormone affects the rate of degradation rather than the sensitivity of a particular site of action.

Further studies of the degradation of labeled barbiturates in normal, castrated and testosterone treated castrates are contemplated in the hope that the particular reactions affected by the hormone may be identified and the hormonal influence on their rates may be determined.

### Tyrosine oxidation

Extracts of rat and rabbit liver acetone powders have been shown to contain a series of enzymes which oxidize tyrosine to acetoacetic acid and  $\text{CO}_2$ . Some of the cofactors required in this series of reactions have been identified. The first step is apparently a transamination reaction converting tyrosine to p-hydroxyphenylpyruvic acid. Ascorbic acid and glucoascorbic acid stimulate the further oxidation of p-hydroxyphenylpyruvic acid whereas pyruvic acid inhibits.

### Biosynthesis of epinephrine

The enzyme system responsible for the conversion of L-phenylalanine to L-tyrosine has been isolated from the liver of animals and humans, partially purified and studied. It is a soluble, labile system requiring both oxygen and pyridine nucleotide for activity.

Tyrosine decarboxylase activity has been demonstrated in beef, adrenal medulla homogenates.

Beef adrenal medulla can convert tyramine to hydroxytyramine by virtue of its ascorbic acid content. This conversion is apparently non-enzymatic.

### Studies of biochemical mechanisms involved in the metabolic transformation of drugs

A possible role of ascorbic acid in the body has been indicated by its action on drugs and other organic compounds.

Ascorbic acid in the presence of oxygen can oxidize non-enzymatically a number of aromatic compounds to yield substances identical with metabolites produced in vivo. The rate of this in vitro oxidation is tremendously amplified by the addition of  $\text{Fe}^{++}$  together with versine (tetraacetoethylenediamine). The following reactions which take place in vivo, can be carried out efficiently by the ascorbic acid,  $\text{Fe}^{++}$ , versine system:



1. Quinoline ---- 3-OH quinoline
2. 1 - 3 dimethylxanthine ---- 1 - 3 dimethyluric acid
3. Acetanilide ---- p-OH acetanilide
4. Tyramine ---- hydroxytyramine
5. Tyrosine ---- dihydroxyphenylalanine
6. Phenylalanine ---- tyrosine

The oxidation cannot be accounted for by the production of  $H_2O_2$  since  $H_2O_2$  acts neither quantitatively or qualitatively in the same manner.

#### ® SECTION ON CELLULAR PHYSIOLOGY

After several months of organizational activity, during which the acquisition of personnel, equipment, and space were of most immediate importance, the Section on Cellular Physiology has reached a stage of relatively efficient research operation. Four main research programs have been initiated and will be described briefly below.

The research activities of the Section may be grouped under the general heading of biosynthesis. Studies on protein synthesis, cholesterol degradation and synthesis, the metabolism of two-carbon compounds, and on the enzymatic details of energy production and utilization in mitochondria and muscle are under way.

#### Studies on the biosynthesis of proteins.

Proteins may be synthesized from amino acids either directly, by an "all-at-once" template condensation of activated amino acid units, or by a step-wise synthesis of peptide intermediates followed by the ordered coupling of these intermediary units. To test the actuality of one or the other of these two possibilities, experiments have been carried out employing a combination of radioactive tracer and enzymatic techniques. In these experiments, radioactive egg albumin has been synthesized, in vitro, using as labeled precursors,  $Cl^{14}O_2$  or C-14 tagged alanine. By means of controlled proteolytic digestion, peptide fragments have been split off from the parent protein molecule. Alanine or aspartic acid has then been isolated from these peptides and the specific radioactivity of these amino-acid residues, derived from the different points along the protein chain, has been determined. The fact that marked differences are observed strongly suggests that intermediary peptide precursors are formed and subsequently coupled during the course of protein synthesis.

As an important adjunct to these studies, an effort has been made to develop micro methods for the separation, determination, and radioactive counting of peptides and amino acids. Such methods are now available and are being applied to the complex mixtures of peptide



material obtained on proteolytic digestion of the radioactive proteins under investigation. These methods employ paper chromatography and micro colorimetry as the basic operations and are designed to yield accurate data with quantities on the order of 20 - 50 micrograms.

Previous experiments have indicated that glutamic acid is not incorporated into proteins as the free amino acid but in the form of an activated precursor not in equilibrium with the amino acid itself. These preliminary studies suggest that a direct carboxylation of some four-carbon acid may lead to the active form of glutamic acid. Studies are under way to test this hypothesis, using C-14 labeled succinic acid and glutamic acid as tracer substances. By study of the location of labeled carbon in protein glutamic, following incubation of tissue slices with the precursors mentioned above, a direct examination of this process is being made.

#### The microbiological degradation of steroids.

A serious difficulty in the study of sterol biosynthetic mechanisms has been the degradation of the steroid molecules, following the incorporation of labeled precursor, or proposed precursor, substances. Since certain mycobacteria can grow on synthetic media with cholesterol as the only source of carbon, an investigation of the enzymatic machinery of these specialized cells has been initiated. The immediate purpose of this investigation is the isolation of enzymes from the ruptured cells which might be used as analytical reagents for specific attack on individual rings and carbon atoms in the sterol nucleus.

The oxidation of cholesterol by cell-free extracts in the presence of 0.1 M cyanide has been achieved. The addition of cyanide appears to cause the accumulation of intermediate compounds and makes possible considerably larger yields of these substances. Preliminary isolation attempts have yielded at least six intermediary keto-steroid compounds which are being examined by infra-red spectroscopy.

The enzyme responsible for the conversion of cholesterol to  $\Delta^4$ -cholestenone appears to be present in large amounts in the cell extracts and its purification and characterization is now under way.

#### Biosynthesis of activated two-carbon compounds and the utilization of these compounds in general biosynthetic compounds

It has become increasingly evident that the basic structural unit for much of cellular biosynthetic activity is the two-carbon fragment, probably acetyl-coenzyme A. Certain aspects of the origin and metabolic role of this substance are being investigated in this Section, using as biological material certain microorganisms particularly rich in coenzyme-A and the enzyme transacetylase.

Transacetylase has been undergoing purification in this laboratory during the past months. Its purity has been increased approximately 125-fold in a fairly simple alcohol-salt fractionation procedure.

Studies have also been initiated on the oxidation of acetaldehyde, a particularly effective precursor of "active" two-carbon compounds. The production of acetyl phosphate from acetaldehyde by C. Kluyveri appears to involve coenzyme-A, DPN, glutathione, and an unidentified acid and alkali labile factor present in boiled extracts of bacteria and beef-liver.

The oxidation of glycolaldehyde is also being studied since this substance may be an important precursor in pentose synthesis. Oxidation of glycolaldehyde is coupled with phosphorylation and a product, tentatively identified as glycolyl-phosphate, has been isolated.

Finally, a study has been made of the process of acylphosphate inter-conversion. The results are consistent with a mechanism, involving co-enzyme-A, in which the energy in one acyl-phosphate molecule is utilized, through intermediate formation of an acyl-co-A complex for subsequent transacylation and phosphorylative cleavage.

#### Enzymes of oxidative phosphorylation.

The major portion of energy available for bodily activity is derived from phosphorylative reactions coupled with electron transport during biological oxidation. Much of this essential activity takes place in mitochondria. Studies on isolated mitochondria and mitochondrial extracts by members of this Section have shown that an important regulatory mechanism in the gearing of oxidative and phosphorylative activity may be the enzyme ATPase. This enzyme appears not to be an apyrase, but a true ATPase whose activity, in conjunction with that of myokinase, may constitute an important factor in the utilization of oxidative energy. Of particular interest is the profound inhibitory action of ADP on this enzyme.

The lipoprotein nature of the ATPase is also under investigation since the nature of the bound lipids and the mode of binding itself may have an important bearing on the functioning of enzyme.

#### Characterization of crystalline glutamic acid dehydrogenase.

This enzyme has been prepared in crystalline form and subjected to numerous physical and enzymatic tests. Diffusion constant and sedimentation constant determinations indicate a molecular weight of approximately 2,000,000. The Michaelis constants for various components of the test system have been determined. The pH optimum, effect of inhibitors, and co-enzyme binding affinities have also been investigated. The enzyme is at present being used to catalyze the conversion of  $\alpha$ -keto glutaric acid to 1 - ( + ) glutamic acid as the last step in the synthesis of C-14 labeled glutamic acid.

### Projects Concluded:

The relative specific radioactivity of aspartic acid isolated from different points along the chain of  $C^{14}$  labeled egg albumin has been determined and supports the "step-wise" hypothesis of protein synthesis described above.

Transacetylase has been purified, approximately 125-fold, to a point where the enzyme can be used for metabolic studies without danger of incidental enzyme activities from contaminating impurities.

Liver mitochondrial ATPase has been demonstrated to act as an important regulatory substance in oxidative phosphorylation.

L ( + ) glutamic acid dehydrogenase has been crystallized and characterized.

### SECTION ON CHEMISTRY OF NATURAL PRODUCTS

The laboratories of the Section on Chemistry of Natural Products were completed during 1950-1951 to an extent which permitted organization of a professional staff and establishment of the first research projects of the Section. The general plan of organization of the Section at the present time involves the formation of two research groups which function independently in the direction of research projects, and which cooperate generally in the broad aspects of the work and function of the Section.

The research programs, under Doctor<sup>®</sup> E. C. Horning and under Doctor B. Witkop, are summarized in Part A; this work was initiated in 1950-1951. The professional staff consists of recent graduates coming from first-rank research groups in organic chemistry in the country. A permanent staff will be built up gradually, and it is expected that flexibility will be maintained by keeping a number of openings for very promising young people, which will be filled through temporary appointments. A small but continuous flow of good young people through the Section will provide a source from which permanent appointments can be made. Close contact with established workers whose interests are of particular significance to the work of the Section would be helpful, and Visiting Scientist appointments may be of assistance in achieving this end.

The growth of the Section, with an increase in research potential, will continue during 1951-1952. It will be necessary to establish a research colony, at the University of Maryland, in order to complete the staffing of the Section in preparation for the move into the Clinical Center. During this period certain new research activities, started in the spring of 1951, will be broadened and modified as conditions may require. A cooperative project with the Section on Chemical Pharmacology, under Doctor Brodie, has been instituted; this work is concerned at present with a fundamental study on adrenergic blocking agents, and it is expected that this work will be expanded into a synthetic program which



will complement the program on drug action of the Section on Chemical Pharmacology.

Two laboratories, one for microanalysis and one for instrumental analysis, have been planned in part around the work of the Section on Chemistry of Natural Products and in part as cooperative laboratories to complement where necessary the work of other Sections of the National Heart Institute. A start has been made in the organization of these laboratories. A microanalyst has been added to the staff of the Section, with the arrangement that all work will be carried out on a temporary basis in the quarters of the Institute for Arthritis, Rheumatism and Metabolic Diseases. Apparatus requirements are now being met for this laboratory. The instrumental laboratory is in operation, with the exception of a recording ultraviolet spectrophotometer which is expected in July, 1951. The personnel and work of this group will be adjusted to fit the needs of the Institute and the Section as they develop. The importance of instrumental methods in modern chemistry is constantly increasing.

Although several important research projects were initiated in the Section during 1950-1951, certain major features of the proposed program of the Section will not be evident until 1951-1952. An investigation of the background and evidence for activity of a number of plant materials of reported therapeutic activity is in progress. After evaluation of the available evidence, a number of these problems will be selected for further study; a brief summary of some of these is contained in Part B. It is also expected that compounds from animal sources which are of interest because of their activity or biological significance will be brought under investigation. Some of these problems will require large scale preparative or isolation work, and facilities for this will be organized as the program develops.

Continuous assessment of individual projects or programs is provided by two types of reports from the staff. A system of monthly technical reports has been instituted, following the general practice of organic chemical laboratories, and a summary semi-annual report is prepared by each professional staff member covering his or her entire program.

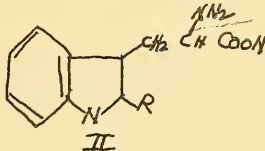
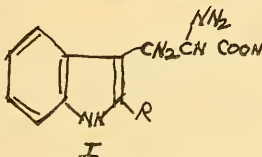
#### A fundamental study of biological oxidation.

An investigation of the various pathways of chemical oxidation of heterocyclic compounds and of certain types of aromatic compounds may be expected to contribute new information about the part of oxidative change of these compounds in biological systems. This broad program has started with investigations of specific oxidation reactions. The indole system, which is present in tryptophane, and in a number of alkaloids, may be oxidized by ozonization, by peracids, and by a variety of other oxidative reagents. In the case of 2-phenyl-3-methylindole, the reaction with ozone provides a stable ozonide. This is quite unusual, and the fact that it is stable provides an opportunity to study further stages of the degradation



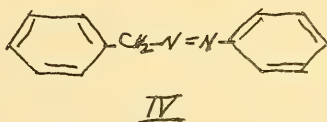
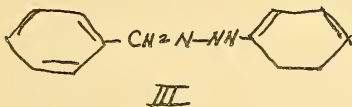
in ways that are ordinarily not possible. Reactions of this compound with reducing agents, with acids, and with organometallic compounds have been investigated. This work, which is nearly completed, provides new information about an oxidative pathway for indoles.

Another oxidative pathway for indoles may be found in the transformation of tryptophane into N-formylkymurenin. This oxidation, which probably proceeds through a hydroxy- or hydroperoxyindolenine, is an oxidation reaction occurring in biological systems, and its investigation is difficult because of the synthetic problems which are involved. Attempts are being made to prepare tryptophane substituted in the 3-position (I) with groups which will allow a study of the oxidation to be carried out without complications due to reactions of the indolenine form (II). Synthetic



work on this problem is well advanced, but the desired compounds have not yet been secured, and no conclusions can be made now.

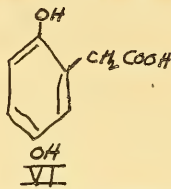
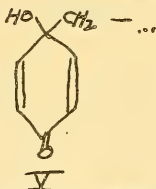
To supplement this work, a study of the oxidation of certain phenylhydrazones has been undertaken. These compounds (III), which may react in tautomeric form (IV), are oxidized by peracids to a monomeric compound containing one additional oxygen atom. This open-chain system is related



to the indolenine form of tryptophane, and there may be a connection between the mode of oxidation in these cases. Since the structure of the oxidation product of III is unknown, the first step which is required is a study of the structure and reactions of this material. This part of the general problem is well under way, but no final decision about structure may yet be proposed.

The oxidation of heterocyclic or aromatic systems through the formation of nuclear hydroxy-substituted compounds is an important pathway of biological oxidation. One such oxidation is represented by the conversion of tyrosine to homogentisic acid (VI), which almost certainly occurs by way of an intermediate containing structure V. The existence of this

intermediate structure may be inferred from present knowledge of the chemistry of related compounds and from metabolic studies with labeled

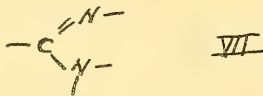


tyrosine. A demonstration of the nature of this oxidation and subsequent rearrangement presents a difficult synthetic problem which is under study; a simple model has been made and several methods for obtaining the desired intermediate compound are being investigated.

A series of studies on the oxidation of the indole system in tetrahydrocarbazoles was undertaken some time ago. The rearrangements encountered in this series were particularly interesting, since they resulted in ring expansion and contraction. An extension of this study in the harman and yohimbine series has been initiated. Attempts will also be made here to obtain derivatives of yohimbine which may possess hypotensive activity.

#### Structural studies on amidines.

The amidine group (VII), represents the nitrogen analog of an ester,



but these compounds possess marked basic properties. This structural group now appears to be present in several classes of alkaloids, and it may be that the significance of the amidine group in relation to physiological activity has not been fully appreciated. This investigation will include the preparation of model amidines, the development of diagnostic methods for the group (VII), and studies on the determination of the structure of aspidospermine and the curare group.

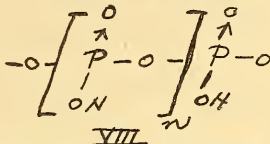
#### Synthesis of serotonin.

Serotonin is an indole derivative with pressor activity and a determination of its structure may throw some light on essential hypertension. From the evidence available at present it appears likely that this compound is a derivative of 7-hydroxytryptamine, and the problem is being

investigated by synthetic means. A new method, suitable for the preparation of 7-hydroxy-indole, has been worked out, and its application to this problem is under investigation.

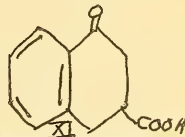
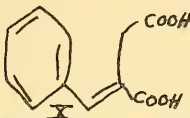
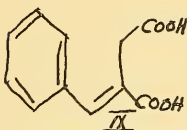
### Polyphosphoric acid cyclizations.

Polyphosphoric acid (VIII), a commercially available form of phosphoric acid, may react as a phosphorylating agent, and as an unusually effective agent in certain cyclization reactions involving acidic or

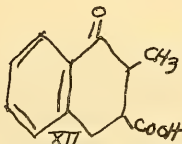


enolic functional groups. Two studies of reactions of polyphosphoric acid have been initiated and completed; one was successful, and while the results in the second study are not fully interpreted, the general results were not the desired ones.

The first study was concerned with the cyclization of arylitaconic acids (IX) and the corresponding benzylsuccinic acids X. Attempted



cyclization of IX and its derivatives led only to the acid anhydrides, although it is known that these compounds may be cyclized to naphthols by pyrolysis. The acids X were cyclized by polyphosphoric acid to the tetralones XI, and a comparison was made here with the action of sulfuric acid. The best method was extended to the preparation of XII. This compound will have Vitamin-K activity, if the body is capable of dehydrogenating the tetralone ring.

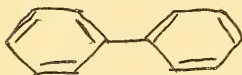


The second study was concerned with the cyclization of a variety of compounds to yield benzofurans, and was directed to the synthesis of compounds related to khellin. In each case the reaction evidently proceeded

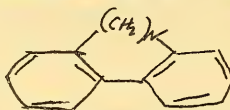
either by general decomposition or by intermolecular reaction. Except for a few supplementary experiments, this investigation of benzofurans has been terminated. A secondary set of experiments, may be correlated with this benzofuran study.

### Compounds influencing cell growth.

A fundamental study of the structure of biphenyl (XIII) and related polycyclic systems (XIV), carried out by Doctor E. C. Horning in 1949-1950, indicated that a paired relationship existed between XIII and XIV (n-3), and XIV (n-1) and XIV (n-2). The stereochemical and resonance

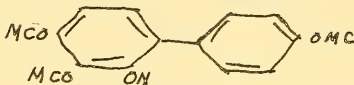


XIII

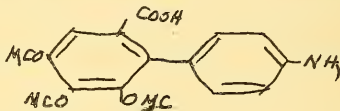


XIV

pattern relationship existing for the first pair was not expected, and it led to a fundamental study of the effect of substituents in XIII and XIV (n-3) with respect to (a) ultraviolet spectra, and (b) antimitotic activity. A number of compounds, such as XV and XVI, are being prepared for examination of physiological action by Doctor Gelhorn of Columbia



XVI



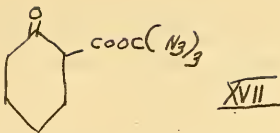
XV

University. The path of this investigation will probably be determined by the results obtained by Doctor Gelhorn, but it is hoped that a general correlation arrived at by means of chemical methods (ultraviolet spectra) will lead to a correlation of physiological activity with structure in this series.

### The Bardhan-Sengupta synthesis.

This project has not passed beyond preliminary stages. It is a continuation of a general study carried on earlier on a reaction method which would be exceedingly useful for the synthesis of polycyclic systems related to natural products, if it could be made to work for cases beyond the first one or two members of each group. A thorough study has already eliminated the Kon modification from consideration, and the present study is based upon the assumption that compound XVII can be alkylated, and that the product can be hydrolyzed easily (by acidic cleavage, rather than by true hydrolysis). This material, XVII, has never been made, and the





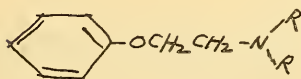
present aim is to accomplish this synthesis by simple methods.

#### Isolation of glycyrrhizic acid.

This project involves the isolation of glycyrrhizic acid from licorice. It has been reported that cures of Addison's disease have been effected by licorice extracts, and by ammonium glycyrrhizate. The active principle is a triterpene glycoside, as far as is known, and this work is directed to the preparation of pure samples of this acid and the aglycone, glycyrrhetic acid. Further chemical work will not be carried out if physiological activity is not encountered.

#### Adrenergic blocking agents.

This project was initiated recently as a joint project with Doctor Brodie, to complement work in the Section on Chemical Pharmacology. It is concerned with the synthesis of agents which will represent variations in certain chemical and physical properties (particularly fat solubility) and which will be designed to provide fundamental information on the mechanism of action of these agents. Two compounds have been made (XVIII, XIX) to provide a start on the program, and further work will be guided by the pharmacological results.



XVIII      R = Et-  
XIX         R =  $\phi$ CH<sub>2</sub>-

### SECTION ON METABOLISM

#### Studies of the renal injury of choline deficiency.

It has not been possible to influence the renal lesions with certainty by denervation of the kidneys or by drugs which specifically affect the autonomic nervous system. Protection was afforded by subcutaneous injections of dibenamine or formaldehyde, apparently due to the local irritation and production of an "alarm reaction." ACTH afforded slight protection, and cortisone afforded protection of the same order

as was produced by the irritating injections. Thiouracil prevented the lesions in almost all of the animals, and this effect was abolished by simultaneous administration of thyroid extract. It appears, therefore, that the lesions are definitely influenced by certain alterations in the endocrine system.

It has been observed that when 0.5-0.6% aureomycin is added to the choline-deficient diet, almost complete protection against the renal lesions results. The ceca of the animals receiving aureomycin become greatly enlarged and filled with semi-fluid material. Administration of aureomycin apparently does not result in morphological alterations in the thyroid or other endocrine glands. It seems likely that the protective effect of aureomycin is associated with alterations in bacterial metabolism in the gut.

Studies of filtration rate, renal blood flow, TMPah and TMglucose in dogs showed no definite change in these functions which could be attributed to the omission of choline from the diet. In connection with these experiments, a progressive decline in TMglucose associated with a decline in filtration rate but not related to the choline content of the diet was noted in two dogs. It seemed that these changes might have resulted from closing down of some of the glomeruli. However, when the filtration rate was increased by rapid saline infusions, there was no increase in glucose TM.

Blood pressures have been determined on a group of rats with chronic renal injury as a result of a period of choline deficiency several months previously. None of these rats has been observed to have a distinctly elevated blood pressure. This is at variance with the results from several other laboratories and perhaps warrants further investigation of influence of diet, strain, etc.

#### Studies of the effects of copper deficiency.

Puppies placed on demineralized water and a purified diet containing about 2 micrograms of copper per gram of (dry) food developed lameness after 1 to 2 months. The lameness was accompanied by anemia, poor growth, graying and poor condition of the hair, thickening and scaling of the skin. Control animals (litter-mates) on the same diet with added copper remained entirely normal.

The symptoms were perhaps exaggerated but not qualitatively changed by simultaneous deficiencies of other trace elements (Co, Mn, Zn).

In the early experiments, it was assumed that the lameness was due to neuromuscular disturbances and resulting changes in the joints. The lameness was sometimes accompanied by wasting of the muscles particularly

about the hips and thighs but the tendon jerks remained apparently normal. In later experiments it became obvious that the disturbance was at least in considerable part due to involvement of the bones. The leg bones became bowed, and the joints large, knotty, and unstable. Many animals developed spontaneous fractures. X-rays have shown striking abnormalities in the bones (chiefly the bones of the extremities) of the deficient animals with thinning of the cortices, absence of trabeculae with ground-glass appearance, and fractures and pseudofractures (Milkman - Looser Zones). There have also been changes at the epiphyseal junctions suggestive of but not entirely characteristic of rickets.

The deficient animals have shown plasma copper levels of 20-30 micrograms percent compared with a normal level of about 100 micrograms percent. Other chemical determinations on plasma have shown no difference between deficient and control animals: Calcium 10-12 mg percent, inorganic phosphorus 7-9 mg percent, alkaline phosphatase activity 9-20 Bodansky units, carbon dioxide (arterial) 19-21.5 vol. percent, pH 7.22-7.38, potassium 4.5-5.0 m. eq. l.

No evidence has been obtained so far of any disturbance in calcium metabolism to account for the bone changes.

The anemia in the copper deficient animals has usually been normo-chronic and normocyte. The volume of packed red cells has fallen to 10 mm or below in several animals. Reticulocytes are reduced in number, platelets sometimes increased in number, and white cells normal.

Autopsy of the animals has revealed few abnormalities in organs other than the bones. Parathyroids appear normal. No renal stones or ectopic foci of calcification have been observed. The cortices of the long bones appear thin and lack trabeculae. The marrow appears normal or possibly hyperplastic.

About two-thirds of the deficient animals have shown the disturbance described. About one-third of the animals have shown little or no anemia and lameness, presumably because stores of copper in the liver and other tissues had been built up by eating appreciable amounts of food other than milk before the deficient diet was started.

Administration of copper to deficient animals has been followed by a prompt and striking reticulocyte response and regression of anemia, changes in hair, and general symptomatic improvement. Fractures heal and cease to occur, though the x-ray changes in bones following therapy with copper remain to be studied.

## SECTION ON CARDIOVASCULAR HEMODYNAMICS

### Experimental program in animals.

The study on vitamins necessary for the maintenance of peripheral hemodynamic mechanisms has shown that:

The dietary intake of vitamin C profoundly influences the ability of the animal (guinea pig) to withstand hemorrhagic shock. Deficiency in ascorbic acid is associated with early death and shock, with a reduction in effective bleeding volume, and inability to produce renal vasotrophic humeral material. This data we have recently published.

Administration of folic acid to albino rats is accompanied by the presence of gross and microscopic renal lesions and the development and maintenance of a persistent and significant blood pressure elevation. The significance of this finding, with respect to the metabolic role of folic acid in such blood pressure increase, is not clear. The close functional interplay that has recently become evident in the literature, among such factors as folic acid, ascorbic acid and Vitamin B12, will make it necessary to carry out rather careful bio-chemical evaluations of the role of each of these factors in the function of such vaso-tonic mechanisms as the adrenal cortex, smooth muscle reactivity to constrictor stimuli, etc. It has become increasingly evident that the study of nutritional factors in hemodynamics is one largely based on the bio-chemistry of metabolism and of essential food factors. The data on folic acid is unpublicized.

The dehydrogenase systems, particularly succinic acid dehydrogenase, in the zona glomerulosa of the adrenal cortex, are profoundly affected by ascorbic acid deficiency.

Histo-chemical studies of the adrenal glands, with the use of the dye, tetrazolium chloride, have shown that such glands closely resemble those which have been inhibited by the administration of desoxicorticosterone to the animal. To our knowledge, this is the first known example of a histo-chemical demonstration of enzyme dysfunction associated with ascorbic acid depletion in the adrenal gland. This material is at present unpublished.

### Clinical program.

The functional and anatomical changes that obtain in the observed capillary bed of the conjunctiva in hypertensive people, will shortly be published in the Journal of Clinical Investigations. This work has uncovered a dynamic pattern in the capillary vessels of such individuals, that was not observed either in normotensive subjects or in patients with normotensive diseases.



The findings have greatly interested the department of Obstetrics and Gynecology. They have begun a long-term survey on the conjunctival capillary bed during normal pregnancy and during pregnancies complicated by such features as albuminuria, hypertension and frank eclampsia. This survey is being conducted in part in the hypertension clinic.

The clinic devoted to the study of various types of hypertensive disease has continued to operate and has expanded to a moderate extent. The hospital here has assigned an additional physician to the staff during the past 6 months. This clinic has been the source of our observations of the capillary bed in hypertension and has greatly expanded our clinical knowledge of hypertension. With the burden of added patients from the Medical Service and the patients from the Obstetrical service and several infants and small children referred to us from Pediatrics, additional help is necessary and an increase in the number of clinic sessions will perhaps be necessary in the future.

#### SECTION ON KIDNEY AND ELECTROLYTE METABOLISM

##### Mechanisms of fluid and electrolyte retention in experimental preparations in dogs.

Effect of adrenalectomy on experimental ascites in the dog with constricted inferior vena cava. Two dogs have been subjected to (1) unilateral adrenalectomy, (2) constriction of the inferior vena cava, and (3) removal of the second adrenal. During the interval between stages (2) and (3), these dogs in every respect behaved as those with constriction of the vena cava alone. Following adrenalectomy and removal of supportive therapy with cortisone and desoxycorticosterone, the animals develop a progressive diuresis which is associated with complete loss of ascites over a period of 10-12 days. At the end of this period, the animals remain in excellent general condition with no fall in arterial or venous pressure and with an increase in renal blood flow and filtration rate associated with an increase in plasma protein concentration. Thereafter, the animals develop the usual signs of adrenal insufficiency including a 50% drop in renal functions. The low sodium-high potassium pattern of fecal electrolyte excretion disappears following adrenalectomy. At present, an attempt is being made to evaluate the rate of turnover of sodium in the untreated adrenalectomized dog with ascites when maintained in a steady state by refeeding the electrolyte lost in the urine.

##### The distribution of electrolytes in tissues.

The standard procedure for evaluating the distribution of electrolytes between extra- and intracellular fluids is based on the assumption that chloride is exclusively extracellular. However, the inulin space

has more recently been advanced as a more exclusive value for the volume of extracellular fluid. If the inulin space is considered to be the extracellular fluid, then some 30% of the chloride of the body must be within cells. There is a need for comparison of chloride and inulin spaces in individual tissues as well as for a more extensive evaluation of the significance of the inulin space.

The problem of the determination of inulin in whole tissues (liver and muscle) is under examination. The major obstacle in this regard is chemical blank which varies with tissue, species and individual animal. The resorcinol method with preliminary yeasting gives better results than either the same method without treatment with yeast or the diphenylamine method with yeasting. Adsorption of the blank on various materials has been attempted. Only diatomaceous earth has had any effect and this has not been reliable or particularly effective. The blank in rat muscle varies from about 3 to 10 mg.% of inulin equivalent per 100 gm. of wet muscle.

A simple, reliable method for constant intravenous infusion of the unanesthetized, unattended rat (for periods up to 16 hours) has been developed. Collection of urine over timed periods through an indwelling catheter and serial blood samples are feasible. The same animal may be used for repeated studies. Using this procedure, the inulin clearance of the rat has, for the first time, been measured by a procedure analogous to those customarily used in man and in the dog. A somewhat higher value for the rate of glomerular filtration in the rat than that determined by other methods has been found. The data should provide a basis for evaluating the reported correlation between filtration rate and urine flow in this species.

#### Methods for evaluation of renal function.

The observation that the clearance of inulin averages some 6-7% higher than that of creatinine in the dog has been confirmed and extended. Further data on the binding of creatinine on plasma proteins have been collected and support the view that the discrepancy between creatinine and inulin clearance is attributable to this phenomenon. Inhibitors of certain tubular transport mechanisms (carinamide and benemid), reported to modify the creatinine-inulin clearance ratio in man, have been found to have no effect on this ratio in the dog.

Initial studies of the relationship of plasma inulin concentration and inulin clearance in man have been done with the view of re-evaluating the early observation that there is no correlation between the two. The fact that this observation has been questioned leaves open to skepticism the entire basis for the measurement of glomerular filtration.

The tubular mechanism for transport of glucose in patients with renal glycosuria.

A series of patients with renal glycosuria is under observation by a group of physicians at Georgetown University. The cooperation of our group in an analysis of the mechanism of this defect has been solicited. To date, one patient has been studied and was found to have a marked reduction of glucose transport capacity (60% below the average normal) as well as a markedly splayed titration curve permitting the excretion of appreciable amounts of glucose at plasma concentrations far below those required to saturate the transport mechanism.

The relationship between respiratory variation in acid-base balance and urine acidification.

It is well known that respiratory acidosis leads to excretion of an acid urine despite deviation of the plasma bicarbonate and vice versa. No quantitative studies of this relationship are available nor has the mechanism by which it is brought about been clarified. Studies aimed at clarification of the phenomenon have been initiated. The production of respiratory alkalosis by electrophrenic respiration has been attempted. Immediately after implantation of the electrodes, reasonably satisfactory hyperventilation can be induced. However, chronic preparations rapidly lose their response to stimulation. Satisfactory respiratory acidosis has been produced by the inhalation of CO<sub>2</sub> mixtures. However, the excitement associated with the hyperventilation makes satisfactory experiments with conscious dogs impossible. In the case of both acidosis and alkalosis further progress depends on the discovery of an anesthetic agent which will not interfere with renal function. Chloralose appears to be the most promising but difficulty has been encountered because of the hyperreactivity which it produces. Further exploration is contemplated.

The nature of the effect of mercurial diuretics on potassium excretion.

This controversial subject is under re-examination as a result of further observations in connection with the relationship between urine acidification and potassium excretion (see projects completed). These observations support the contention that mercurials reduce potassium excretion from high levels by inhibiting tubular secretion of this ion. The increase produced by mercurials when potassium excretion is initially low is not satisfactorily explained. The explanation that is due to inhibited reabsorption does not appear satisfactory. The hypothesis under examination at present is that (1) tubular secretion of potassium is in large part dependent on the load of sodium delivered to the mechanism, (2) the increase due to mercurials is simply a matter of increased electrolyte load presented to the secretory mechanism, (3) a certain amount of exchange of potassium for sodium can proceed without that active mechanism which is inhibited by mercurials. No change in effect of mercurial has been observed as dosage is increased from 0.5 to 3.0 ml. of salyrgan and the independence of



potassium excretion and filtered load during mercurial diuresis has been reconfirmed, when changes in filtered load are due to moderate changes in filtration rate.

The effects of plasma volume and colloid osmotic pressure on the excretion of electrolytes and water.

The diuresis which follows injection of albumin solutions has been found to be primarily a water diuresis followed secondarily by an increased excretion of electrolytes. The effect is produced by isosmotic, iso-oncotic solutions but not by hyperoncotic solutions. The effects have been attributed to inhibition of secretion of posterior pituitary antidiuretic hormone but changes in renal hemodynamics have not been adequately excluded. Further exploration of the phenomenon with particular regard to changes in glomerular filtration is in progress. It is possible that only with diabetes insipidus animals will final solution of the problem be feasible.

Mechanisms of fluid and electrolyte retention in experimental preparations in dogs.

Constrictive pericarditis: Pericarditis has been produced by implantation of cellophane in the pericardial cavity. Results obtained by application to the entire heart are similar to those produced by covering the right heart only. The clinical syndrome produced may be divided into two phases -- (1) an acute phase of fluid retention in which generalized edema occurs and in which there may be pulmonary congestion of a severe degree; (2) a chronic phase in which fluid accumulation is largely in the form of ascites. All animals develop the acute phase. During this period, sodium and chloride are almost quantitatively retained, despite a fairly striking increase in glomerular filtration rate and renal blood flow. The venous pressure becomes elevated. Several animals have died in this acute phase showing evidence at autopsy of marked pulmonary congestion. The acute phase, in those animals which survive, is terminated by spontaneous diuresis. Several animals have thereafter remained free of evidences of circulatory insufficiency. The majority, however, enter a chronic phase characterized by the formation of ascites as rapidly as permitted by the sodium intake. During this period, the filtration rate returns to the control level or, if protein depletion results from loss of nitrogen in ascitic fluid removed, to a level well below the control. Venous pressure is continuously elevated in this period but shows marked fluctuations with the volume of ascites; following abdominal paracentesis, there is a sharp drop in venous pressure (jugular as well as femoral) to levels which may fall as low as 120 mm. H<sub>2</sub>O, well below the value (160 mm. H<sub>2</sub>O) usually associated with the onset of fluid accumulation. Such drops in venous pressure, are not, however, accompanied by diuresis. The electrolyte excretion in the stool shows a pattern of low sodium, high potassium concentrations suggestive of adrenal hyperactivity. It seems fairly certain that lowered glomerular filtration is not an important factor in the fluid retention in this preparation. Beyond this, the explanation for the lowered salt excretion remains uncertain. A report of this work is being prepared for publication.



Constriction of the thoracic portion of the inferior vena cava:  
Long-term balance, hemodynamic, and renal function studies are being conducted in dogs whose inferior vena cavae have been constricted by a band placed around the vessel between the diaphragm and right auricle. Soon after the constriction is produced, the femoral venous pressure becomes elevated, there is almost total retention of the dietary sodium, and gross development of ascites is apparent on the fourth post-operative day. There is no drop in glomerular filtration rate initially; any later drop appears correlated with loss of protein in ascitic fluid and can be reversed by reducing the rate of fluid accumulation and increasing dietary protein. As in the animals with constrictive pericarditis, the stool electrolyte pattern is suggestive of adrenal hyperactivity. Study of the eosinophile count has not, however, been found to corroborate this hypothesis.

The relationship between urine acidification and potassium excretion.

It has previously been shown that urine is acidified by a process of hydrogen ion secretion by exchange of hydrogen for sodium ions in the distal tubule and that carbonic anhydrase is involved in the process whereby the hydrogen ions are made available. The process by which potassium is secreted has likewise been demonstrated to be one of ion exchange presumably for sodium in the distal tubule. A relationship between potassium metabolism and urine acidification has been known to exist because of the alkaline urine which accompanies administration of potassium salts, the alkalosis which accompanies potassium depletion, the depletion of muscle potassium which accompanies alkalosis, and the increase of muscle potassium with acidosis. Abolition of urine acidification with a new and highly effective carbonic anhydrase inhibitor results in a very marked increase in potassium excretion. The increment in potassium excretion may exceed the total filtered potassium and hence is due to enhanced secretion. This is confirmed by the fact that the increase in potassium excretion is prevented by mercurial diuretics which, however, do not prevent the effect of the drug on urine acidification. This finding as well as the above mentioned relations between potassium metabolism and urine acidification appear to be dependent upon a competition between potassium and hydrogen ions for some component of the ion exchange mechanism whereby both are secreted. A number of clinical phenomena appear to be explicable on this basis. This work has been submitted for publication and should appear in an early issue of the American Journal of Medicine.

The significance of the urine carbon dioxide tension.

The fact that the  $\text{CO}_2$  tension of alkaline urines may be very considerably higher than that of the plasma has never been adequately explained. Most recently this finding has been attributed to continued secretion of hydrogen ions into a urine containing large amounts of bicarbonate; delay in dehydration of the carbonic acid formed was considered to permit escape

of the urine from the tubules before re-equilibration of the  $\text{CO}_2$  tension could occur. The observation that  $\text{CO}_2$  tensions are not reduced when hydrogen ion secretion is suppressed by inhibiting carbonic anhydrase makes this explanation untenable. The alternative explanation that the high  $\text{CO}_2$  tension is due to mixing of urines of different pH has been subjected to theoretical analysis and experimental verification. It can be shown that any elevation of  $\text{CO}_2$  tension produced by mixing urines of different pH is highly dependent on buffer concentration. Experimentally, it is found that the  $\text{CO}_2$  tension of urines in each limited range of pH's is directly correlated with buffer concentration, confirming the hypothesis that mixing accounts for the observed phenomenon.  $\text{CO}_2$  tensions calculated from  $\text{CO}_2$  content and pH have been found to check well with those determined by direct analysis. No standard correction of urine pH for temperature is practicable. This work is being prepared for publication.

#### SECTION ON TECHNICAL DEVELOPMENT

Development of ultraviolet photofluorometry to include measurement of ultraviolet emission.

To investigate the extent to which substances that have absorption bands in the mid to far ultraviolet exhibit fluorescence in the ultraviolet region. Theoretical considerations suggest that a considerable number of these compounds are fluorescent or could be made to exhibit fluorescent spectra which would be of use in characterization and quantitation.

An indicating and recording fluorescent spectrophotometer utilizing a low aperture quartz microspectrometer as the dispersing element has been assembled and tested as a means of making a rapid survey of compounds and obtaining a photographic record of the emission spectrum as well as reference spectra of the source used.

The flaming carbon arc, the Zirconium arc, the condensed metallic spark discharge, and various mercury vapor lamps were tested and their spectra photographed for future reference. The  $\text{AH}^4$  mercury lamp and the quartz alpine burner proved to give the best lines for reference calibration as well as the most intense lines for excitation of fluorescence. The Iron arc provided the richest source of far ultraviolet radiation but is extremely unstable in intensity.

Photographic and photometric methods of detecting the emission spectra were tested and both found to be satisfactory and the necessary apparatus to utilize those methods was constructed and tested.

Work continues to:

Extend the range and sensitivity of the current apparatus to allow the

photometric type of survey to be applied to compounds having feeble emission or emission in the far ultraviolet.

To set up high aperture monochromators and suitable light sources for fine measurements of fluorescent spectra.

To utilize the apparatus above to examine the fluorescent spectra of compounds of biochemical interest and determine the value of these spectra in relation to characterization and quantitation.

An incidental finding to date is that the fluorescent spectrum analyzer in its present form can be effectively utilized as a means of quickly selecting filters for photofluorometry.

Diodrast method for measurement of residual volume and stroke output of the heart.

To determine the residual volume, stroke volume, and minute output of the intact human heart. The method is to inject Diodrast into the right ventricle by means of a cardiac catheter and to record the rise and fall of Diodrast concentration in the pulmonary artery. The X-ray transmission through the pulmonary artery is a function of the Diodrast concentration within the artery. This transmission may be recorded by a special form of the Electrokymograph. By suitable calibration procedure, the concentration of Diodrast in the pulmonary artery vs. time may be obtained. From this curve, the residual and stroke volumes and minute output may be calculated.

It was first demonstrated that records of pulsatile density changes of the pulmonary artery could be made with the standard electrokymograph. Preliminary mathematical considerations indicated that an injection of 15 cc. of 70 percent Diodrast into the human right ventricle would produce easily measurable changes in X-ray transmission through the pulmonary artery.

The electrokymograph was modified to provide the necessary d.c. amplifications and integration of the pulsations, required to produce a reasonably smooth curve of appropriate amplitude.

In collaboration with Doctor Richard Bing at Johns Hopkins Hospital, sample curves were obtained from two intact human subjects. These curves indicated the feasibility of the method, provided that a suitable calibration scheme could be devised for converting the X-ray intensity transmission vs. time record to a Diodrast concentration vs. time record.

A mathematical method for this calibration has been determined and is now in process of being tested.



## Multichannel photographic recorders.

To develop multiple channel galvanometer type photographic recorders suitable for the study of laboratory problems and physiological phenomena. This is in part a continuation project and in part a new project.

One recorder capable of at least 18 simultaneous channel recordings has been completed. It has been in almost daily use for physiological recordings during this past year. By request, this machine has now been moved to the animal surgical room for use by the Section on Kidney and Electrolyte Metabolism.

A second similar recorder is being build for use primarily by our own Section. This machine will be much smaller physically, on wheels, and will be capable of at least six channel simultaneous recordings. For maintenance and convenience purposes, all galvanometers, light sources, timer, etc., for the two machines will be interchangeable.

One part of this project on multichannel records is completed. One recorder capable of at least 18 simultaneous channel recordings has been completed. By request, this machine has been moved to the animal surgical room for use by the Section on Kidney and Electrolyte Metabolism.

Development of apparatus for recording the relative cross-sectional area of the total cardiac silhouette.

To record the cross-sectional area of the total cardiac silhouette as viewed under the fluoroscope.

A conversion for the electrokymograph was designed which provides true d.c. response in the measurement of total transmitted X-ray intensity within the shutter frame of the fluoroscope. The heart was framed out by the shutter, so that the output from the device varied with changes in the cross sectional area of the heart silhouette.

It was recognized that variation in the intensity of the primary beam would be indistinguishable from variation in the area of the silhouette if only one pickup tube was used. To eliminate this source of error, another photo-tube was set up to look through the chest exclusive of the heart, and the output to the recorder was the difference between the outputs of the two tubes. Errors due to variations in primary beam intensity were thereby eliminated.

Valsalva experiments were performed on several normal subjects. It was soon found that the variation in X-ray transmission through the lung field with respiration was considerable and adversely affected the performance of the system. The reference cell was then placed to look at the primary beam directly, while the viewing cell remained unchanged. Several acceptable Valsalva records



were then obtained. In one record, a large line voltage transient occurred. No effect was observed on the record. The design of this instrument has been transmitted to Dr. Robert Grant. The instrument is to be constructed at Baltimore.

Modification of a Cambridge electrocardiograph.

To so modify and improve the apparatus that it will record three simultaneous electrocardiographic leads.

The modifications on the Cambridge ECG have been completed. Three ECG leads can be simultaneously recorded. The instrument is in current research use at the U.S.P.H.S. Hospital.

Upon request of Dr. J. A. Southworth of the U.S.P.H.S. Hospital, a pump was designed to circulate blood around one of the heart chambers in order to allow the chamber to be approached surgically.

The heart pump was constructed in accordance with the design, by a contractor. It is in use and found to perform satisfactorily in experimental dog surgery.

Of incidental importance, it has been found that variation of density of the lung field with respiration is considerable.

#### SECTION ON GERONTOLOGY

The effect of intravenous administration of sodium lactate on PAH Tm in humans.

An investigation of possible intermittency in nephron activity in the human.

The effects of intravenous administration of lactate on the acid base equilibrium of the blood.

The relationship of renal plasma flow, glomerular filtration rate and PAH Tm to basal oxygen consumption.

Age differences in water and electrolyte distribution in the field compartments of the body.

Age differences in adrenal cortical response to surgical procedures.

The dietary calcium requirement in aged males.

Age differences in responsiveness of peripheral blood vessels to standardized stimuli.

Age differences in decrement in performance following continuous work tasks.

An over-all synthesis of data on age changes in renal function has been made. It is concluded that age changes can be explained primarily on the basis of vascular alterations and it is suggested that extra-renal factors may dominate the control of the renal circulation, particularly in the aged. A decrease in Tm glucose with age has been demonstrated. The recent decrement in glucose Tm is approximately the same as the per cent decrement in resorptive Tm (PAH or diodrast) previously reported. Experimental evidence for tubular resorption of hemoglobin in the human kidney has been obtained. It has also been shown that the intravenous administration of sodium lactate results in a significant increase in PAH Tm in humans. Control experiments lead to the conclusion that the observed effect is due to alterations in the tubular transport mechanism for PAH. An acute transient rise in Tm PAH and Tm glucose follows the intravenous administration of aminophyllin and pyrogen. Since both these agents have been shown to increase renal plasma flow, the possibility of perfusion of previously inactive nephrons in the human kidney is considered.

A technique for the simultaneous determination of blood volume, extracellular fluid space and total body water has been standardized. Preliminary results indicate a reduction in total body water in the aged. The intravenous administration of 200 meq. of r-lactate was followed by an average increase in serum pH of 0.05 and an average increase in serum bicarbonate of 3.7 meq. These changes in the acid base equilibrium of the blood were of the same order of magnitude as those produced by an equivalent amount of sodium bicarbonate. The renal plasma flow, glomerular filtration salt and Tm diodrast were more closely related to basal oxygen consumption than to surface area of the body in a group of 30 subjects. It has also been shown that the decrement in basal oxygen consumption with age is small beyond the age of 40 years.

More precise estimates of oxygen required for physical exercise have shown that the over-all efficiency is significantly less in aged subjects than in young. Unexplained is the finding that at very slow rates of work efficiency (average 9%) is markedly lower than at moderate rates of work (average 15%) in old subjects.

The previously reported observations on the effect of chronic administration of ACTH has been confirmed in additional subjects. The cardiovascular responses to chronic administration of ACTH (rise in venous pressure, increase in heart size, and increase in pulse rate after standard exercise) were greater in old than in young subjects. Preliminary results on the dietary requirements of Ca in aged subjects indicate that at intakes of 100 mg. Ca per day, negative calcium balances occur. It is also clear that when calcium is added to the diet, readjustment to positive Ca balance occurs within the first week in aged subjects as well as young.

Preliminary results on three subjects have shown that following surgical repair of hernias old subjects show a prolonged period eosinopenia.

Using a new method, developed in this laboratory, the relationship between transmission velocity and arterial pressure has been evaluated in the intact radial artery in approximately 55 subjects. In most subjects the experimental data fit the equation  $v = ap^b$  from which parameters (a and b) for individual subjects may be calculated. In the cases which deviate from this relationship, the slope of the velocity-pressure curve changes markedly and  $\frac{d \log v}{d \log p}$  continues

to decrease with increasing pressure. The radial artery does not behave as an ideal elastic system. Preliminary results indicate that in the radial artery, changes in distensibility-pressure relationships with age are in the opposite direction from the results of previous conventional studies which have considered elastic behavior to be present.

A preliminary instrument designed and built by the Technical Development Section to make possible a study of attenuation and other characteristics of pulse wave transmission as a function of frequency and pressure was tested. It was demonstrated that with this instrument oscillating waves of pressure could be superimposed on the arterial pressure and transmitted along the artery. In its present form, the displacement provided by this instrument varied considerably with frequency and with the amount of loading. In the absence of any provision for monitoring the motion developed by the instrument, relative constancy of input amplitude could not be obtained. The results of these tests were summarized and transmitted to the Technical Development Section. On the recommendation of the Technical Development Section, the instrument was returned and the collaborative project was discontinued.

In an effort to isolate the factors involved in the reduction in speed of performance observed in older subjects, measurements of the velocity of nerve conduction were made. Preliminary results indicate that speed of conduction in peripheral nerves is significantly slower in old subjects than in young. A study of decrement in mental and psycho-motor tasks with continuous work periods was also made. Although the initial rate of work was slower in the aged than the young subjects, the aged showed significantly less decrement with continued work than did the young.

A comprehensive "A Classified Bibliography of Gerontology and Geriatrics" was completed and published in book form by Stanford University Press.

#### Concluded:

The metabolic and physiological responses of aged and young subjects to the chronic administration of ACTH.

The effect of intravenous administration of lactate on Tm PAH in man.

Age changes in glucose Tm.



## SECTION OF GENERAL MEDICINE AND EXPERIMENTAL THERAPEUTICS

This Section, located at the U. S. Marine Hospital, Baltimore, Maryland, completed its first year of formal existence. Though a research team was working in the field of cardiovascular disease at the Marine Hospital, Baltimore, these activities did not become a part of the National Heart Institute until January 1, 1950. Consequent to this development many changes naturally ensued during the year and others are still in progress. Many members of the previous professional staff had been recruited on short term commitments. There was also a lack of experienced research workers. It was necessary to expand gradually as proper personnel could be recruited, and as satisfactory laboratory space and equipment could be made available. It appears that the early organizational problems have been solved and the Section is now an operating research team.

Laboratory and Ward Space. The total number of beds available to the Section has remained approximately the same during the year, with approximately 60 beds devoted to this program. Many of these beds have been utilized for the care of routine Public Health Service beneficiaries with cardiovascular disease, but those demands have not been great enough to prevent the admission of any research patient. Beds have always been available for the admission of patients for research purposes regardless of whether such patients were normal Public Health Service beneficiaries or otherwise.

One of the serious limitations of the Section to date has been an inadequate amount of laboratory space, but this problem is largely solved at present. The hospital has recently made additional space for two small and one large laboratory and this space is now being fitted with laboratory benches and other necessary equipment. By trading space with the laboratory of the National Cancer Institute the Section has been able to move all of its other laboratories to the same floor and thus consolidate related facilities into one compact unit. The Section presently includes three large and four small laboratory rooms on the third floor of the Laboratory Building for chemical procedures and other related work.

Training Activities. The training activities of the Section have progressed during the year. Regular teaching ward rounds, seminars and conferences have been conducted. The pertinent teaching conferences of the Medical Service and the general hospital staff have also been utilized for instruction. A detailed course in electrocardiographic interpretation was presented by the members of the Section during the year. The staff members of the Section have also attended the National Heart Institute Seminars held at Bethesda. These activities have been important, especially for the training of the younger members of the staff. They have also been valuable for the teaching experience gained and for the cohesive spirit which they engendered in the personnel of the Section. It is contemplated that this group will be called upon for significant teaching duties in the Clinical Center and this experience is assisting the development of a team capable of assuming such responsibilities.



Research Activities: Individual investigator reports of research projects for the year have been submitted. The following discussion is intended to present a brief summary of this work and to point out some of the more significant results.

During the year eighteen separate research projects have been initiated. Thirteen of these projects have been in the field of medical problems, while five projects have been of a surgical nature. The projects fall into four general groups. This has largely been occasioned by the training and research interests of the individual investigators.

Hemodynamic problems include the analysis of factors concerned with the control of heart size, various technical phases of electro and vector cardiography, the study of the mechanism of postural hypertension, and acute hemodynamic response of dogs to vena caval obstruction and infusion.

A few projects have been related to experimental therapeutics. Examples of this type of study are: clinical use of cation exchange resins in the control of edema, experiments with mercurial diuretics and the evaluation of drugs in the diagnosis and treatment of hypertension.

A few studies have been conducted with relation to the metabolic aspects of cardiovascular diseases. Studies on the effects of desoxycorticosterone glucoside on renal function in dogs, and the relationship of the adrenal cortex to hypertension, are examples of studies in this field.

Several projects of a surgical nature have been conducted. Investigators have studied the cardiac output in ventricular fibrillation, various methods of preserving arterial grafts, an extra corporeal circulation, and diagnostic aortography. Another investigator has studied methods of exteriorization of the vesical trigone in dogs to facilitate renal function studies in such preparations.

The number of significant findings to date have been relatively few. Many of the projects have been in progress for such short periods that conclusive results have not been possible. Others have involved such extensive laboratory support that they had to be curtailed or postponed. Still others have required extensive exploration and development of techniques or instruments and are still in the formative stages. However, the following brief summary of interesting or significant findings can be presented:

Normal humans can tolerate massive salt loading by oral route up to 10% of body weight in 8-12 hours without serious functional embarrassment. The excretion of salt and water in these individuals follows a

fairly characteristic pattern and the patients are essentially back to preloading status within 24 hours.

A method for presenting the "electrical contour" of the body in EKG studies has been developed and is being subjected to experimental application.

Marked diuresis and weight loss with associated negative sodium and chloride balance can be produced in a cardiac patient by means of a cross-linked, polyacrylic carboxylic acid cation exchange resin.

The antihypertensive effects of present veratrum preparations appear quite limited. Parenteral administration produces transient results with considerable side effects. Oral preparations used were relatively ineffective in the doses tolerated by patients.

Procaine amide appears to be of little value in the treatment of auricular rhythm disturbances.

The Soffer technique of study of adrenal cortical function as applied to essential hypertension does not demonstrate any adrenal-controlled electrolyte disturbance similar to that seen in Cushing's disease.

Cross circulation experiments in dogs indicate that the operative procedure is feasible and that this method may be a valuable development in experimental surgery for the purpose of obtaining a bloodless heart for operations within the chambers.

A small ventricular output continues during short periods of ventricular fibrillation in dogs. The systemic venous pressure rises sharply with the onset of ventricular fibrillation and is sufficient to produce a large volume of blood flow if a low pressure reservoir is attached to the pulmonary circuit. Successful defibrillation chiefly depends upon an adequate supply of oxygenated blood to the coronary circulation.

Vially preserved arterial grafts may be preserved up to six months and appear superior to non-vially preserved grafts. Formalin preserved grafts may be used for arterial grafts with better than 80% success. Satisfactory arterial grafts may be artificially produced by fibrous reaction to implanted plastic tubing.

The canine vesical trigone can be exteriorized successfully in a high percentage of animals and greatly facilitates renal function studies in these animals.

Only five of the projects initiated have been concluded. They are:

Exteriorization of the Canine vesical trigone  
Study of ventricular fibrillation  
"Soffer test" in essential hypertension  
The use of pronestyl in auricular fibrillation  
Hemodynamic response of dogs to vena caval occlusion and infusion.

#### SUMMARY AND RECOMMENDATIONS

It is recommended that the Section of General Medicine and Experimental Therapeutics continue its operation at the U. S. Marine Hospital, Baltimore, in a manner similar to that in operation during the past year.

Personnel requirements of the Section appear adequately provided for in plans which already have been developed.

Adequate laboratory and clinical space are available to the Section. No further developments in this direction are anticipated within the next year.

The research activities of the Section are developing slowly but satisfactorily. It appears that only time is necessary for this work to gain momentum.

#### PUBLIC HEALTH METHODOLOGY

Doctor Frederick G. Gillick, Chief, Heart Disease Control Section, was appointed to a staff position in the Intramural Research Branch, on December 1, 1950, to serve liaison between the research aspects and control program of the Public Health Service, and investigate on request procedures, devices and instruments which are under consideration for use in the control program.

#### ACTIVITIES

Initiated a preliminary investigation of the flicker fusion - nitroglycerin test. Report made on March 15, 1951 and sent through channels to the Division of Chronic Disease and Tuberculosis. The test was not recommended for use in public health programs. Reaction to the report and comments regarding format were requested of the Division of Chronic Disease and Tuberculosis.

In March 1951, requested \$1,500.00 from Division of Chronic Disease and Tuberculosis for assistance in investigation of ballistocardiograph. In May 1951, \$1,000.00 was made available. A table model has been ordered, and a loan of a portable model has been accomplished. The instruments are

to be placed at the Marine Hospital at Staten Island. Simultaneous recordings from the portable and table models are to be done on normals and a variety of pathological cases to ascertain the comparability of the records obtained by the two types of instruments as well as to determine reproducibility of findings with those appearing in the literature. This is being done with a view to ascertaining the possible value of taking routine ballistocardiographs at Framingham as well as to its possible value in public health programs.

On being transferred to National Heart Institute, supervision of work on the Heart Sound Recorder developed by the former Division of Chronic Disease was continued. This instrument was placed under Doctor W. Proctor Harvey at Georgetown University Hospital. In order to develop its teaching and follow-up potentialities, a research grant application was submitted.

Work has continued on the analysis of the electrokymograms of the Framingham project. A report of activity to date on this study follows. Doctor Bert R. Boone is collaborating in this study.

Assistance in writing the section on heart disease for the new book on Military Preventive Medicine is continuing.

Study of Approximately 3000 Electrocardiograms.  
A Progress Report.

Studied 1000 records to discern type of left ventricular patterns most common for purposes of setting up a reading guide on pattern basis.

Set up pattern guide.

Read independently 100 Ekg's on basis of pattern guide  
Agreement was as follows:



Dr. Gillick -- Table I

Boone	Norm.	N-→ D	D-→ N	D	D-→ Abn.	Abn.	Unsat.	
Normal	26	6	4	2				38
Normal -→ Doubt	7	5	4	1				17
Doubt -→ Norm	1	1	1	2	1			6
Doubtful	2		1	2	5			10
Doubtful -→ Abn.	2		1	6	5	3		17
Abnormal					1	5		6
Unsatisfactory	2	1					3	6
	40	13	11	13	12	8	3	100

O/O Agreement	Definition of Agreement
47	Identical impression
83	<u>±</u> 1 Category on the scale of 6.

Read records mentioned in (3) and placed them in 6 categories -- Normal (N), normal to doubtful (ND), doubtful to normal (DN), doubtful (D), doubtful to abnormal (DAB), and abnormal (AB). The categories were based on the following items: disturbances of rhythm, left ventricular pattern in the various positions, characteristics of the "iso-metric relaxation" period, and general appraisal of the Eky.

Readings made in the above were analyzed against reported Framingham diagnosis. --The following results were obtained:

Table II

Clinical Diagnosis	Combined EKY Evaluation (excluding unsatisfactory)						Average Evaluation
	Norm. (o)	Norm. → Dbt. (1)	Dbt. → Norm. (2)	Doubt. (3)	Doubt. → Abn. (4)	Abnormal (5)	
No. CVD	26	10	5	6	6	6	$\frac{92}{59}$ 1.7
Def. RHD	14	1	1	4	5	3	$\frac{50}{28}$ 1.8
Ques. RHD	7	2	1	1	3	0	$\frac{19}{14}$ 1.4
Def. CAD	6	3	1	7	8	4	$\frac{78}{29}$ 2.7
Ques. CAD	5	4	3	1	1	0	$\frac{17}{14}$ 1.2
HCVD	9	3	5	2	5	0	$\frac{39}{24}$ 1.6
Ques. HCVD and all other with 140/ or 90/ or Both.	19	12	2	4	8	4	$\frac{80}{49}$ 1.6
	86	35	18	25	36	17	217

The tendency towards abnormal EKY for the definite CAD group is significantly different from the No CVD group in a statistical sense but of questionable fractional value. All other disease categories don't differ significantly from No CVD.

Re-examination of several of the records examined in (4) and (5) above - in light of complete record (exclusive of X-ray film) led to the following:

Studied microfilm on limited number of Framingham subjects. Classified the cases on the basis of definite cardiac disease or definitely no evidence of cardiovascular disease. The definite cardiac disease cases were further classified on the basis of whether in our judgment from the record the individual was presently incapacitated - i.e. having difficulty performing his or her ordinary tasks and those in whom there was apparently no functional capacity. Segregating these last groups was hazardous, nevertheless, if the pattern form of left ventricle was related to functional capacity it was felt

some clue could be obtained. If no trend or difference could be obtained, then it was felt this was probably a blind alley. With limited numbers of cases to choose from the group studied is small. The results were as follows:

Table III

Category I (No CVD - "Good Function*") (10 Cases)	N (0)	ND (1)	DN (2)	D (3)	DAB (4)	AB (5)	Average Evaluation
	4		2		1	3	2.2
Category II (CVD - "Good Function*") (10 Cases)	5		2	2		1	1.5
Category III (CVD - "Poor Function*") (6 Cases)	1		1	2	2		2.7

\*Function - Opinion based on examination of microfilm record of Framingham data. Good if no obvious present cardiovascular interference with routine activities.  
Poor - if obvious present CVD complaints apparently incapacitating for routine work.

As the next step - we are presently pulling all Eky's exhibiting so-called "paradoxical" motion in certain positions. The individual's records will be examined for a possible common denominator. Should certain findings appear to be common -- these findings will be tested on other samples of the 3000 records to determine differences of significance if any. To date, electrokymograms of 79 individuals were found to have "paradoxical" motion in one or more of the following left ventricular positions: MLB, LLB, and MLB-ROA. Table IV shows the breakdown of these 79 cases by age group, sex, and the presence or absence of cardiovascular disease. Detailed examination of the Framingham records on these cases is the next step.

Table IV

Age Groups	Total	Total	Males				Total	Females			
			CVD	CVD ?	No CVD /	No CVD		CVD	CVD ?	No CVD /	No CVD
20 - 24	1	1				1	0				
25 - 29	3	1				1	2	1			1
30 - 34	11	7*		2	2	2	4				3
35 - 39	10	6	2	1		3	4*	1	1		1
40 - 44	11	8		2	1	5	3			1	2
45 - 49	7	4	1			3	3	1			2
50 - 54	18	10	4	3		3	8	2	1	1	4
55 - 59	12	6	2	2	1	1	6	2	2	1	1
60 - 64	3	3	1	1	1		0				
65 - 69	3	1	1				2	2			
	79	47	11	11	5	19	32	8	5	4	14

\* One male and one female not included in breakdown because no Dx at this time.

? Diagnosis - states that No CVD is present, but X-ray on other statements are made which does not leave CVS above suspicion.

/ No CVD - Other disease states such as diabetes, anemia, tuberculosis, emphysema, etc. may be mentioned.

#### GRANTS AND TRAINING BRANCH

The Grants and Training Branch is responsible for the administration of five programs, providing support of investigators, graduate students, under-graduate students in medicine, and institutions. These programs are designed to stimulate, and in part support, advances in ultimate control of heart disease through research and the improvement of the education of workers in this field.

The work of the Branch is concerned with the review and ultimate approval of the several types of grants which comprise this program. It is organized with a Chief and Assistant Chief, who comprise the entire professional personnel of the Branch, and a clerical staff of eight people. The Branch is responsible for performing all of the services connected with the processing of grants, including



the arrangements for the three meetings of the National Advisory Heart Council each year. The latter responsibility includes the transcription of a verbatim record of Council proceedings and the preparation of the final minutes.

The professional staff maintains relationships with various professional people concerned in the large number of grants which the Branch handles. The Branch also maintains a complete record system which contains detailed information for the guidance of the Council and of the administrative staff regarding scientific and fiscal aspects of the several programs.

Several changes have been made in the operation of the Branch during the fiscal year. With the appointment of a new Chief, the organization of the Branch was modified and adjustments were made in its physical quarters to provide for more convenient and efficient flow of work. A number of changes were made in the administrative detail with the result that the several work peaks incident to the Council meetings were reduced and the clerical work load was distributed more evenly throughout the year. There has also been a significant reduction in the over-time required of the staff without an increase in full-time services.

Security of original documents was arranged through the microfilming of all records of legal and financial importance. A fire-proof safe was obtained for the storage of these records, together with the original Council verbatim minutes and sound scribe recordings of the meetings. A number of files were eliminated and the storage of old records was markedly reduced with a gain in effective working space. Reorganization of the information-recall system was undertaken and is still in progress. However, delays in securing special filing equipment have made it impossible to complete these changes, which will enable the Branch to furnish information more easily on the detailed aspects of the grants programs without the addition of new personnel.

Reporting Council meetings is a new function. It has been accomplished with relatively little difficulty by combining the duties of the secretary to the Chief and the reporting function into a single job, requiring only an adjustment in the grade of the position. Although this increased the salary of the secretarial position, the increase was much less than the cost of the reporting service from other sources. The meetings were previously reported by the Division of Research Grants, and probably there has been no transfer of funds to adjust for this factor. As a result, there is a slight increase in cost of the Grants and Training Branch operation because of this item.

Among the physical improvements in the Branch are a number of items which have improved the efficiency of clerical operations. These include the use of an increasing number of office machines (most of which were items not in use elsewhere in the Institute). The most important single change in the physical surroundings has been the arrangement whereby the secretary who cares for the detail of the smaller programs is located in an office which is also used as a

conference room for the Branch. This was accomplished by consolidating the office of the Chief and Assistant Chief. The conference room is the most heavily used unit in the Branch. The combination of filing space, conference room, and secretarial space has worked much better than description would indicate. Other units in the Heart Institute have found the conference room helpful and have used it to expedite their work.

#### Research Grants Program

The National Heart Institute awarded 404 research grants to investigators in the cardiovascular field during fiscal 1951. These grants amounted to \$4,472,436. 528 grant applications were received aggregating \$6,627,147 in requests for support. Of these 419 were approved by the National Advisory Heart Council in the amount of \$5,227,513. The Surgeon General approved 404 grants referred to above.

The research grants program of the National Heart Institute is similar to the programs of the other categorical institutes of the National Institutes of Health. The objective is to stimulate and give financial support to studies of cardiovascular diseases by research scientists in the Nation's educational institutions, research laboratories and hospitals.

Projects most likely to provide better fundamental understanding of heart disease, or which will lead to immediate practical advances in its cure or control, are selected by panels of outstanding scientists. While primary consideration is upon the promise each proposal gives for sound and rapid advances, the emphasis in evaluation constantly shifts as the results of investigations become available and promising leads come to light.

Accomplishments: A continuous growth of medical knowledge with respect to the major causes of heart ailments is providing an indispensable foundation for the development and application of new methods and techniques for the control, treatment and prevention of these conditions. The ultimate understanding and subsequent elimination of congenital heart defects, rheumatic fever, essential hypertension, coronary thrombosis, congestive heart failure and arteriosclerosis is dependent on the progress recorded in research laboratories.

Significant advances in both fundamental and clinical research supported by the National Heart Institute include the following:

#### Rheumatic fever

Administration simultaneously of massive doses of the hormone agents, ACTH and cortisone, to treat rheumatic fever and prevent residual heart damage where possible.

Basic studies of the body's sensitivity reaction to "burned out" streptococcal germs as possible clue to origin of rheumatic fever.

Development of new and better salicylates to counteract symptoms of rheumatic fever (fever, pain, soreness of joints).

### Congenital heart disease

Experimentation and development of new surgical techniques for correction of defects.

Establishment of artery graft "banks" and development of better preservative media for keeping grafts longer.

Development of plastic substitutes for heart valves and blood vessels.

### Essential hypertension

Studies of chemical factors in kidneys found experimentally to raise blood pressure.

Studies of enzyme systems which govern metabolism of substances produced in kidneys and liver (VEM & VDM) that influence circulation disturbances and high blood pressure.

Joint physiological, biological, biochemical and psychological studies of essential hypertension to clarify degree emotional stress and personality pattern play in condition.

Further studies of the rice and low-salt diets -- (a) to improve the diets; (b) to acquire better understanding of how they achieve their effect in body.

Development of new and better drugs to counteract soaring pressures. These are of two types: (a) vasodilators, which dilate constricted vessels and permit freer flow of blood; (b) chemical sympathectomies, which block nerve impulses to vessels.

Continued research in surgical sympathectomies, the cutting of important nerve beds that control the elasticity reactions of blood vessels.

Further development of cation exchange resins. Plastic substances which remove salt from ingested food, preventing its absorption by the body.

### Coronary disease and arteriosclerosis

Development of a "cold" spray to freeze excruciating pains of coronary thrombosis. (Pains often send patient into shock, seriously interfere with treatment measures.)



Important studies which show use of oxygen in coronary thrombosis limits area of muscle tissue damage.

Discovery that injured muscle area caused by blood clot blocking circulation pours off toxic poison which can send heart into serious fibrillation (convulsions) and death. Studies under way to develop counteractant drugs.

Discovery that spleen produces two important hormones that regulate clotting of blood in the body. Uses seen for cases of bleeding due to radiation sickness, as anticoagulant to prevent further clotting in coronary attacks, and in cases of hemorrhage due to over-dosage of anticoagulant.

Studies of how the body burns its fats, such as cholesterol, for possible clue to hardening of the arteries and coronary artery disease. Cell metabolism, which underlies cholesterol changes in the blood, studied for possible clue.

Studies which indicate blood vessel walls are more than tubing for carrying blood, with indications they play active role in breaking down and building up important cell foods, including cholesterol and other fatty acids.

Continued studies of the Sf 10-20 giant cholesterol-bearing molecule for its significance and bearing on hardening of the arteries, and its possible usefulness as a test for the condition.

Development of new surgical procedures to increase circulation to heart muscle in severe cases of coronary artery disease. (Use of grafts and pasting section of stomach called omentum to heart to channel in greater supply of blood.)

### Congestive heart failure

Studies of role of hormones in salt and water balance, upset in heart failure.

Studies of role of liver in regulating activity of adrenal and sex hormones, believed to control salt and water retained in body.

Nutritional studies of heart failure patients, who despite excessive weight often (due to water accumulation in tissues), lack important proteins and amino acids.

Improvement and development of existing and new diuretic drugs for relief of water-logged tissues.

Kidney studies for clue to origin of condition.

ACTH and cortisone studies as they affect condition.



Instrumentation and otherwise

Development of mechanical heart now being supported in six institutions.

Development of mechanical kidney.

Ballistocardiograph studies of heart action now widely studied.

Cineangiocardiology -- motion picture photography of heart in action, as seen on a fluoroscopic screen. Technic being perfected. Will aid study and diagnosis of congenital and acquired heart diseases.

Electrokymography, widely used for research studies but undergoing refinement.

Three-dimensional vectorcardiograph.

Rehabilitation

Research on new and better techniques and methods.

STATUS OF HEART RESEARCH GRANTS, BY SUBJECT-CATEGORY  
June 1, 1951

<u>Category</u>	<u>ACTIVE</u>	
	<u>No. of Projects</u>	<u>Amount</u>
Arteriosclerosis	37	621,517
Congenital Heart Disease	5	32,719
Congestive Heart Failure	21	271,765
Coronary Heart Disease	6	47,115
Electrocardiography	10	110,963
National Blood Program	6	205,800
Hypertension	51	665,291
Miscellaneous	48	509,673
Pericardial Disease	0	---
Peripheral Vascular Disease	14	109,530

STATUS OF HEART RESEARCH GRANTS, BY SUBJECT-CATEGORY (cont'd)

June 1, 1951

<u>Category</u>	<u>ACTIVE</u>	
	<u>No. of Projects</u>	<u>Amount</u>
Pharmacology	19	137,283
Physiology	106	1,043,400
Public Health	0	---
Pulmonary Circulation	4	77,131
Roentgenology	10	172,561
Rheumatic Fever	11	148,171
Subacute Bacterial Endocarditis	1	12,204
Surgery	31	327,374
Thrombosis and other Blood Studies	<u>10</u>	<u>71,233</u>
Totals	384	\$4,563,730*

Does not include 29 Fellowship Supply Grants in the amount of \$15,642

\* Does not include 9 National Blood Program Grants approved at June meeting and paid from 1951 Funds in the amount of ..... 81,319

\* Includes Active 1950 Grants in the amount of ..... 188,255

DISTRIBUTION OF CURRENT RESEARCH GRANTS BY AGE OF PRINCIPAL INVESTIGATOR

<u>Age Bracket</u>	<u>Number of Investigators</u>	<u>Total Amount granted</u>	<u>Size of Average Grant</u>
25-29	5	\$ 29,921	\$ 5,984
30-34	39	464,216	11,903
35-39	84	896,667	10,674
40-44	95	1,026,472	10,804
45-49	51	640,022	12,549

DISTRIBUTION OF CURRENT RESEARCH GRANTS BY AGE OF PRINCIPAL INVESTIGATOR (cont'd)

<u>Age Bracket</u>	<u>Number of Investigators</u>	<u>Total Amount granted</u>	<u>Size of Average Grant</u>
50-54	49	\$ 738,243	\$15,066
55-59	36	494,655	13,740
60-64	22	264,713	12,032
65-69	2	23,174	11,587
70-75	2	17,700	8,850

Construction grants

The construction grant program of the National Heart Institute continued active throughout 1951 in that the original appropriations for construction included a contract authority for fiscal 1951. The program made no new grants in 1951 but merely continued payments on construction projects approved in the preceding year. A total of \$4,459,000 (19 grants) was available for fiscal 1951, although only a small portion of the amount was actually paid within the time period stated. A report on the progress of the construction of research facilities for work in the cardiovascular field was made at the June 1951 meeting of the National Advisory Heart Council. This indicated that several smaller projects had been completed and that the bulk of the projected facilities were actively under construction and would be in use by calendar year 1953. The execution of these construction grants has been hindered by the shortage of critical supplies, especially steel, and in one or two instances by physical difficulties related to increasing prices and the institutional contribution to the construction planned. This most important program is unlikely to become active in the immediate future due to the national emergency and the governmental policy that non-emergency construction must wait until our emergency needs are met.

Grants for improvement of undergraduate medical education

The Grants and Training Branch presented 55 grant applications for the support of undergraduate medical education to the National Advisory Heart Council during fiscal 1951. Of these, 46 grants to undergraduate medical schools which have participated in the program in previous years were approved and the funds made available. The program of grants to medical schools is assisting the development of a strong comprehensive course of instruction in the cardiovascular diseases by enabling the medical schools to pay for personnel and facilities necessary to improve and expand their teaching curricula relative to heart diseases.

Due to the present low level of support of such a program (which is considered by the National Advisory Heart Council to be minimal), it was felt that no additional schools could be added as the aggregate funds for this item were exhausted by the participating schools. The present level of grants is \$8,000 maximum for a school of basic medical sciences, which provides only the first two years of medical education, and \$14,000 maximum for four-year medical schools which award the M.D. degree. It was determined during fiscal 1951 that all the medical schools in the United States, with the exception of one in Texas, were desirous of participating in the program. Formal applications were not invited due to the evident inadequacy of funds available and the poor prospect that these could be effectively distributed to include additional schools.

#### Research fellowship grants

106 fellows who were in training for work in the field of cardiovascular research were supported by the National Heart Institute in fiscal 1951. The fellowship program was chiefly administered by the Division of Research Grants of the National Institutes of Health, with the Grants and Training Branch of the Heart Institute being responsible for the selection of the persons to be trained through its Fellowship Board. Total funds expended for heart research fellowships were \$300,548.

Through awards under the fellowship program the Heart Institute is assisting young scientists to devote full-time to their development as research workers and to direct their interests into the cardiovascular field. Operation of the program is thus adding a new and more highly trained group of younger scientists to the research potential of the nation at a time when there is a demand for such personnel. This group will continue to represent an invaluable asset for the furtherance of cardiovascular research in the future.

Success of the Public Health Service research fellowship program is indicated by the fact that a recent survey showed that 92 percent of former research fellows are now engaged in full-time or part-time research.

#### Traineeships

One of the smallest but one of most important of the programs administered by the Grants and Training Branch is that which underwrites the training of graduate physicians in newer techniques in the field of cardiology. This program is designed to improve the general level of practice with regard to heart disease by providing general practitioners, young internists, and other physicians dealing with these diseases with the newer knowledge for its diagnosis and management. 48 trainees, selected from the country at large, were supported from fiscal 1951 funds aggregating \$152,600. This important program is entirely administered within the Grants and Training Branch.



TECHNICAL SERVICES BRANCH  
R. C. ARNOLD, CHIEF

FRAMINGHAM HEART DISEASE EPIDEMIOLOGY STUDY

The Framingham Heart Disease Epidemiology Study has as its objective the careful study and evaluation of all the factors which may contribute to the development or the progression of arteriosclerotic and hypertensive heart disease.

To accomplish this purpose, a random selection of adults between the age group, 20 to 59 years, residing in the town of Framingham, Massachusetts, was made. Six thousand, five hundred and fifty-one persons were included in this sample. An attempt is being made to examine all the individuals in this sample group with the hope of doing periodic re-examinations over a twenty-year period during which all the data of life habits, illnesses, etc. believed to be in any way pertinent to the development of degenerative cardiovascular disease will be tabulated. Patients who are found to have heart disease are referred to their own private physicians for appropriate treatment and clinical management.

The work of enlisting the support and active cooperation of the selected individuals has been and is a major undertaking in itself. Concentration on this phase of the project has occupied considerable attention during the past year. The enlistment of selected individuals--in contrast to the acceptance of volunteers--although begun in May 1950, did not get into full operation until September 1950 due to inadequacy of physical facilities. These facilities were expanded to accommodate an examination rate of approximately 3,000 persons per year. When this was completed, the activity of the community organizing was stepped up to bring the selected individuals into the program at approximately this rate.

During the year 2,131 initial examinations and 254 re-examinations were completed. The work of the Health Educator in organizing the community must continue until as great a percentage of the selected individuals as possible is brought into the study. It is hoped this will be done at least by the Fall of 1952.

A preliminary analysis of the work of the committee indicates that they are securing the active cooperation of about 80% of the sample group. When deaths and removals are taken into consideration, it is seen that the actual refusal rate is relatively low.

During the past year laboratory facilities were set up at the Boston City Hospital to permit satisfactory blood chemical determinations. The clinical facilities at the clinic in Framingham were expanded to accommodate a larger number of patients.

Coding of records was begun to permit tabulation of the results of the initial examination at an early date. Microfilming of the records has been completed with storing of copies of the records in two places outside the clinic as insurance against destruction of the records by fire.

A study to determine the effectiveness of a single Lead I, Electrocardiogram has been completed and is awaiting clearance for publication.

Several other similar studies have been started and will be completed during the year.

A paper on the program was presented at the Annual Meeting of the American Public Health Association in St. Louis, Missouri, November 3, 1950 and published in the March 1951 issue of the American Journal of Public Health. Discussion of the program was also presented at the Harvard School of Public Health, at the Massachusetts Symposium on Hypertension, and at the Lawson General Hospital, Atlanta, Georgia.

A preliminary study of the feasibility of carrying out a dietary study has been made and will be influential in making a decision as to whether such a study should be part of the second examination.

A camera to photograph vessels in the scleral conjunctiva under magnification has been added to the study and a ballistocardiogram will become a part of the examination within the year.

As indicated above, the past year has to a great extent been devoted to organization. Although this will continue to occupy our attention for at least another year, it is believed that the coming year should begin to show some results of the study in the form of scientific reports on the data obtained.

#### BIOMETRICS RESEARCH SECTION

During the past year the Biometrics Research Section provided statistical consultation to clinical research studies and to the intramural laboratories; completed the assembling of long-term mortality data for circulatory diseases computed according to current definitions, and undertook preliminary negotiations for conducting direct population studies related to circulatory disease.

Outstanding accomplishments of this Section included:

Mathematical and statistical aid to intramural laboratories in analysis of experimental data and solution of theoretical equations.

Provision of statistical consulting service to the cooperative study on lipoproteins and atherosclerosis including the experimental design and analysis of data for a comparability test of quantitative measurements made by investigators using ultracentrifuges.

Statistical assistance to the cooperative study on the use of ACTH and cortisone in rheumatic fever.

Analysis of heart disease case-finding data based on chest X-ray examination and co-authorship of two papers on this subject submitted to Circulation.

Development of a mechanical processing system for Framingham examination statistics.

Assistance to the Framingham staff on various statistical problems.

Compilation of selected morbidity and mortality data into a reference handbook.

Preparation of a paper on the volume of childhood mortality from "degenerative diseases" as compared with "childhood diseases", submitted to Public Health Reports.

Preparation of material for a public information pamphlet on heart disease statistics.

Appointment of a mathematical statistician as chief of the Section on Laboratory and Clinical Studies.

#### HEART INFORMATION CENTER

A developing program of professional and public information was continued during the year, carrying out provisions of the National Heart Act. Informational services were provided to keep individuals and organizations in the heart disease field informed as to current developments and the experience of others, to promote increased use of present knowledge and the effective application of research findings, and to assist early casefinding, diagnosis, and treatment through lay education.

Activities included the preparation and distribution of heart disease information-education articles, leaflets, pamphlets, reports, and other publications; planning and development of audio-visual materials, exhibits, radio and television broadcasts and transcriptions; maintenance of an inquiry and reference service; and cooperation with other agencies and organizations in planning and executing informational and educational projects relating to cardiovascular diseases.

Publications issued during the year included the National Heart Institute Circular, a bulletin issued at intervals containing information of value to workers in the heart disease field; The National Heart Institute, a handbook for administrative use; Heart Disease--A Story of Progress, a booklet for the general public on the medical advances in heart disease; Heart Disease-Reading for the

Layman, a selected reference list; the Case of the American People Vs Heart Disease, issued by the American Heart Association in cooperation with the National Heart Institute, which contains information for the public concerning heart disease; Abstracts of Research Papers in the Field of Adrenal and Pituitary Glands and Extracts, for researchers working in the general field of pituitary-adrenal physiology, and Proceedings of the First Conference on Electrocardiography, (consisting of 22 scientific papers, an index and a bibliography) sponsored by the National Heart Institute in May, 1950. A series of heart leaflets: Heart Disease, Rheumatic Heart Disease, Hypertensive Heart Disease and Coronary Heart Disease was prepared for publication.

At the request of the National Advisory Heart Council, the Heart Information Center prepared plans for the issuance of a Heart Bulletin for physicians. The Heart Bulletin, jointly stimulated and supported by the American Heart Association, the National Heart Institute, and the Division of Chronic Disease and Tuberculosis, Public Health Service, will be published independently by the Medical Arts Publishing Foundation, a non-profit public service institution which is part of the University of Texas.

In addition, the Heart Institute assisted in the preparation of counter cards, prescription bulletins and editorials for the health information program through the Nation's 16,000 pharmacies. This project was jointly sponsored by the American Pharmaceutical Association, the American Heart Association and the National Heart Institute. The Center also prepared various informational materials for National Pharmacy Week, 1950, dedicated to heart disease which was sponsored by the American Pharmaceutical Association, American Heart Association, and National Heart Institute. The materials included Heart Quiz, a leaflet, and Heart Disease and the Pharmacist, a 2-panel display placard for drug store windows.

The Heart Information Center prepared a film strip on the Electrocardiograph; assisted in the editing and distribution of the motion picture, Report on the Living, on rheumatic fever and ACTH, in cooperation with the Bureau of State Services, Public Health Service; and prepared a motion picture script, Understanding Heart Disease.

In addition, the Center cooperated in the preparation of several national radio and television programs. Plans for a series of radio transcriptions on various types of heart disease are now in progress. Several scientific exhibits were prepared for professional organizations and the general public.



NATIONAL INSTITUTE OF DENTAL RESEARCH

H. Trendley Dean, Director

C O N T E N T S	Page
Introduction.....	103
Research.....	103
Epidemiology.....	103
Oral Biochemistry.....	104
Oral Bacteriology.....	104
Functional Morphology.....	105
NIDR Unit (U. S. Public Health Service Hospital, Stapleton (S.I.), New York).....	106
Dental Research Grants.....	106
Dental Research Training.....	107



## INTRODUCTION

The National Institute of Dental Research was established by an Act of Congress, in June 1948, for the purpose of conducting, assisting, and fostering research pertinent to the cause and prevention of dental diseases and conditions. It includes, at present, an Epidemiology and Biometry Laboratory, an Oral Biochemistry Laboratory, a Functional Morphology Laboratory, a Laboratory on Oral Bacteriology, and a Grants and Training Branch.

Owing to continued shortage of laboratory space at the National Institutes of Health in Bethesda, Maryland, some of the research work had to be carried out at field stations. These included one at the U. S. Public Health Service Hospital at Stapleton, Staten Island, New York; and another at the Eastman Dental Dispensary of Rochester. The Institute also maintains an office at Grand Rapids, Michigan, in connection with the water fluoridation studies.

### R E S E A R C H

F. A. Arnold, Associate Director in Charge of Research

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### EPIDEMIOLOGY

The Grand Rapids-Muskegon study on the effect of fluoridation of water supplies for caries control was continued. The number of examinations made this year was increased to include children in the second, third and sixth grades, in addition to those age groups previously studied. The results of these observations indicate that dental caries has been reduced in Grand Rapids by approximately 65% in the younger school children (5-7 years of age) and by about 20% in the older children (13-14 years of age).

A study was carried out of the fluoride-dental caries relationship in the case of adults who had a life-long history of drinking a natural fluoride-containing water. It was concluded that an inhibition of dental caries, very much like that seen in children, lasted at least through the age of 44 years. The results showed that they had about 60 percent less decayed teeth, and had lost only about one-fourth as many teeth as had similar adults who had been drinking continuously a fluoride-free water.

Working hypotheses were established, for future study, that a diminution of dental caries is not necessarily followed by an increase in periodontal disease; that such disease may be measured by means of a mathematical index; and that current assumptions as to the association between periodontal disease and certain systemic diseases may be in error.

Studies were initiated of the epidemiology of periodontal disease and of the influence of climate upon human response to fluoride in the water supply.

A study was made of the rate of calcification of the carpal bones of children who were reared in areas where the public water supplies contained fluoride in concentrations from about 3.9 to 4.4 ppm F, and the results were compared with those obtained in the case of a control group of children reared in an area where the public water supply was free of fluoride. No essential differences were found.

#### ORAL BIOCHEMISTRY

Experiments were made on the fluoride uptake of vegetables prepared in fluoride-containing water; and other experiments were carried out on the production of caries in rats kept on an essentially cereal diet. Both of these studies are still in progress.

A comparative study was made, using the young growing rat as the experimental animal, of the availability of the fluorine of some compounds for caries inhibition, causing incisor striations, and also with reference to properties of depositing in bones and teeth. The compounds studied were NaF,  $\text{Na}_2\text{SiF}_6$ ,  $\text{Na}_2\text{PO}_3\text{F}$ ,  $\text{KPF}_6$ , and  $\text{CF}_3\text{COONa}$ . The results showed that when given with the drinking water, in a concentration equivalent to 50 ppm F, the NaF,  $\text{Na}_2\text{SiF}_6$ , and  $\text{Na}_2\text{PO}_3\text{F}$  reduced dental caries, deposited fluorine in the bones and teeth, and caused marked incisor enamel striations to essentially the same extent. When, however, these compounds were administered intraperitoneally, none showed any cariostatic effect; but they produced enamel striations and deposited fluorine in the bones and teeth.  $\text{KPF}_6$  and  $\text{CF}_3\text{COONa}$  were physiologically inert insofar as could be indicated by caries inhibition, enamel striations, and deposition of fluorine in bones and teeth.

Experiments were carried out on the effect of age on fluorine incorporation in bones and teeth. With no previous exposure to fluoride young rats incorporated more fluorine in bones and teeth than mature rats. Rats on continuous exposure to 10 ppm F in the drinking fluid incorporated decreasing amounts of fluorine in bones and teeth as they grew older.

In preparation for studies on the mechanism of the reactions between dental enamel and fluorides, a method was developed for the synthetic preparation of hydroxyapatite of a high degree of purity. It was found that with a hydroxyapatite of such purity and working with fluoride concentrations of 0.1 to 2.0 percent, the reaction proceeds mainly with the formation of  $\text{CaF}_2$ , but some fluorapatite is also formed.

#### ORAL BACTERIOLOGY

Extensive studies were made on the oral lactobacillus group. In this connection, there was developed a very selective lactobacillus medium, which has been tested extensively and found to be satisfactory. These studies included the carrying out of repeated lactobacillus counts on saliva samples



from 150 school children, during one year, and accompanied also by clinical and X-ray examinations.

In a study of lactobacillus and streptococcal populations of hamsters, there appeared no relationship between either lactobacilli or streptococci and caries. Problems of sampling are difficult and need further study.

A study was also made of the products of fermentation of the lactobacilli. This work is still in progress.

In connection with studies of rat caries, diets have been found on which the animals develop caries on the buccal and labial tooth surfaces. This type of caries is common in humans and, therefore, it becomes possible to study this type of caries experimentally in rats.

Several antibiotics have been found to be effective in inhibiting the development of occlusal caries in rats. These include, in decreasing order of effectiveness: Penicillin, bacitracin, aureomycin, chloramphenicol, and streptomycin.

A method was developed, which has been used with success, for the preservation of viability and pathogenicity of the virulent Nichols' rabbit strain of Treponema pallidum when dried in vacuo from the frozen state. This work was conducted by an American Dental Association Fellow on duty at the Institute.

#### FUNCTIONAL MORPHOLOGY

Methods have been developed for the preliminary handling and embedding of dental tissues, from which suitably thin sections can then be cut for direct electron microscopic examination. This is an important advance in electron microscopy, because it is now possible to study the ultra-fine structure of teeth in preparations similar to those long used in optical microscopy.

Extensive investigations have been made of the organic framework of mature and also of developing enamel and dentin. A submicroscopic fibrillar network has been found permeating the prismatic and interprismatic substance of mature enamel. This network does not appear to be preformed, but rather to develop during calcification from the originally homogeneous matrix. Preliminary exploratory studies have also been made of thin-sectioned pulp, bone, and periodontal membrane.

Optical and electron microscopic studies of spread and also of thin-sectioned dental cuticular isolates have provided new information about the mode of attachment of epithelium to enamel, as well as about the formation of secondary cuticle. These findings are of considerable value for a better understanding of the structure and properties of tooth surfaces, and as necessary background for studies of periodontal disease.

Replica studies of tooth surfaces have been continued, with particular emphasis on the early manifestations of caries. The effects of certain acids and of new polishing methods on tooth surfaces have also been investigated.

Electron diffraction studies of the permanence, thickness, and significance of the calcium fluoride deposits formed when enamel is treated with sodium fluoride have been continued.

NIDR Unit: USPHS Hospital, Stapleton (S.I.) New York

During this fiscal year, a Research Unit was organized for experimental work in oral mycology and for related studies involving the use of radioactive isotopes. A start has been made on studies of the relationship of pathogenic fungi to diseases of the oral cavity.

In vivo studies were made on the permeability of the tooth to inorganic salts. It was found that ions of iodine and sodium can penetrate the tooth centripetally and pass into the systemic circulation with uptake locally of iodine<sup>131</sup> in the periodontium. Preliminary results indicate that human serum albumin labelled with iodine<sup>131</sup> can penetrate the teeth of cats and enter the periodontium. These studies are still in progress.

#### DENTAL RESEARCH GRANTS

In the fiscal year 1951, thirty-six research projects were supported by dental research grants. Most of these researches were carried out at universities and colleges; and the remainder, in hospital laboratories. These researches included seventeen projects which may be considered as relating to some phase of dental caries and aiming to aid in its ultimate prevention.

Other subjects studied included the etiology of periodontoclasia; relation of vitamin C to periodontal disease; endocrinological factors in periodontal disease; production of periodontosis in the spider monkey; establishment of a quantitative method of indexing gingival disease; histopathology of gingivae in systemic disease; transplantability of salivary gland tumors; effects of experimental denervation and ischemia on teeth in dog and monkey; malocclusion with special reference to heredity; relationship between muscle forces and dental facial anomalies; bone and tooth development as influenced by vitamin B deficiency; relation of developmental factors, other than nutritional, to tooth form in the rat; prenatal influence on development of teeth and supporting structures; cranio-facial growth norms during changing dentition; facial growth; dental pulp reaction to heat; operating characteristics and efficiency of dental handpieces; effect of abnormal muscular tensions upon the sites of growth of the cranium and face; and chemistry of dental silicates.

### DENTAL RESEARCH TRAINING

The purpose of the dental research training is to aid and encourage qualified dentists and other college graduates to enter the field of dental research by providing adequate training in the application of basic sciences to dental research. Since dental research is a comparatively new field, it has been found difficult to obtain qualified personnel to conduct dental research. For prior to the recently awakened interest in dental research, following closely the demonstration of the fluoride-dental caries relationship and the possibility of reducing dental caries through proper adjustment of the fluoride concentration of drinking waters, dental colleges gave little attention to training in research. The result has been that most dental graduates were interested only in practical operative dentistry and lacked sufficient training in basic dental research. Until the dental colleges have had the opportunity and time to adequately fill this need, as many medical schools have already done in the medical field, such a supplementary training program will be highly desirable.

During this fiscal year, there were sixteen active fellows in the field of dentistry. All of these fellows were engaged in research. Their research subjects included the salivary flow in relation to water metabolism and thirst; various aspects of infection with herpes simplex virus; metabolic activities of microorganisms of the oral cavity; influence of endocrine glands on the metabolism of water and electrolytes; metabolic requirements of organisms isolated from the mouths of patients susceptible to dental caries in comparison with those resistant to dental caries; physiological effect of fluorine in the diet of animals upon enzyme systems; relationship between normal oral streptococci and those associated with subacute bacterial endocarditis; electromyographic analyses of certain facial muscles in man; chemical composition of human saliva; effects of systemic diseases upon the oral cavity; protective action of saliva against dental decay; and studies on the periodontal tissue.





NATIONAL MICROBIOLOGICAL INSTITUTE

Victor H. Haas, Director

C O N T E N T S	Page
Introduction.....	111
Research Laboratory Reports.....	111
Laboratory of Infectious Diseases.....	111
Laboratory of Tropical Diseases.....	126
Laboratory of Biologics Control.....	131
Rocky Mountain Laboratory.....	139
Laboratory of Clinical Investigation.....	145
The Extramural Program.....	145



## INTRODUCTION

The National Microbiological Institute - established as an Institute in 1948 - is charged with responsibility for conducting, coordinating, stimulating, and fostering fundamental research in the broad field of microbiology, and for the operation of a national program of biologics control. To carry out such responsibilities, this Institute is organized into Laboratories of Infectious Diseases, Tropical Diseases, Biologics Control, and Clinical Investigation, the Rocky Mountain Laboratory, and an Extramural Programs Branch.

The work program of this Institute during the past year, as in past years, has been characterized by the dynamic having undergone many shifts in emphasis and in approach to meet new problems, new knowledge, and new public health needs. In past decades, microorganisms were significant in terms of death rate and disabling illness; today their great significance derives from their importance in terms of aggregate illness and economic loss. Virus and rickettsial diseases now demand the same heavy emphasis that bacterial diseases required in past years. Bacterial diseases, on the other hand, require a new approach, in the light of current national need, along such lines as developing new and better techniques and more effective application of known techniques to meet hitherto unknown implications that might develop in terms of possible defenses against potential agents of biological warfare. These are the things that make microbiological research dynamic in nature; that make this area of research a continuing function - a function that must be pursued constantly and changed frequently, not only to maintain existing public health gains, but to fill the gaps in neglected and lagging research areas, and to improve constantly and to help maintain the general public health.

## RESEARCH LABORATORY REPORTS

### THE LABORATORY OF INFECTIOUS DISEASES

Karl Habel, Chief

The Laboratory of Infectious Diseases--senior laboratory of the Institutes in terms of program--continues to emphasize a basic research approach to problems of microbiology. During the past fiscal year numerous changes have been made to meet the need for shifts in emphasis or approach in the study of certain infectious diseases or disease agents in terms of current public health, military health, or civil defense significance. Principal program changes, other than reorientation of approach, included temporary termination of research units on infectious mononucleosis, conjunctivitis and sarcoidosis in order to enable the conduct of projects having a higher national priority--namely: laboratory work on infectious hepatitis and serum jaundice; expansion of laboratory research on streptococcal diseases and rheumatic fever; scientific direction and administration of the Regional Influenza Study program; initiation of a Sectional Research

Program; and initiation of a field-laboratory study of endemic influenza including field vaccine evaluation. Specific current and planned research projects will be described below along with the more important accomplishments of the past fiscal year.

## SECTION ON BASIC STUDIES

### Physical Microbiology Unit

Work was continued on a project which deals with the influence of neurotropic viruses on cell metabolism. A special project was initiated on the role of CO<sub>2</sub> presence in mediating the Pasteur effect. These studies, requiring large quantities of tissue, have been carried out mostly with normal mouse liver, and to some extent with certain transplantable mouse tumors. When basic procedures have been worked out, they will be adapted to infected neural tissues which are more difficult to obtain in large quantities.

The data indicate that CO<sub>2</sub> may play an essential role in mediating the Pasteur effect. The results suggest important applications in the study of the metabolism of virus-infected cells.

Work was continued on the development of media and isolation procedures for the more effective separation of subcellular particulates in a morphologically recognizable and enzymically active state. The objectivity of this study is to determine whether the Krebs cycle activity can be obtained on isolated Mitochondria, or structures differentiated from Mitochondria, especially the tagged melanin granules. Comparisons will then be made between mitochondria of normal and virus-infected tissues. Janus Gren B staining and destaining is used as a further criterion for enzymic activity.

Studies on separation procedures are in a beginning stage. The results should be significant in connection with studies of the interrelationships between viruses and mitochondrial elements of the cell.

In cooperation with the Virus Unit, cytological and enzymic studies of normal and Cocksackie virus-infected mouse muscles were begun.

Application of phase microscopy, and vital staining procedures, have led to new information concerning the pathology of Cocksackie virus-infected muscle tissue. Enzymic studies have not progressed to the stage where final conclusions can be drawn.

### Basic Immunology Unit

The study of the effect of ultraviolet radiation upon the reactivity of antibody was continued. Attempts are being made to elucidate the structural changes in the antibody molecule caused by irradiation and to correlate these



changes with the observed changes in the reactivity of the antibody. When antibody to bovine serum albumin is irradiated, a marked change occurs in the precipitin reaction. With a certain degree of irradiation no change in the total precipitable antibody occurs but the antibody is precipitated by much less antigen, the antibody-antigen ratio in the specific precipitates being increased. The antibody becomes more sensitive to the effects of antigen excess, being more readily inhibited by the latter. At the same time the antibody loses its ability to passively sensitize guinea pigs to the appropriate antigens even though the antibody still combines with the antigen. Further irradiation causes the antibody to become non-precipitating though in the presence of untreated antibody it will coprecipitate. It is evident that some structural change has occurred in the antibody as a result of which the antibody no longer causes passive anaphylaxis but still specifically combines with and precipitates the antigen. It appears likely that anaphylaxis is associated with some specific structural attribute of antibody other than that involved in antigen-antibody union. It is hoped that the nature of the structural alterations can be defined and will shed light on the mechanism of anaphylaxis and precipitation and perhaps other manifestations of antigen-antibody union. Ultraviolet absorption spectra of irradiated antibody indicate that irradiation, breaking intramolecular bonds, has caused the antibody globulin to assume an extended configuration.

A study of the antigenicity of desoxyribonucleoprotein was initiated. If it is possible to produce antibodies to desoxyribonucleoprotein it is planned to study the effect of these antibodies, when passively administered, upon the function and morphology of the organs from which the nucleoprotein was obtained.

Preliminary experiments have thus far failed to demonstrate precipitating antibody to desoxyribonucleoprotein, however, the unusual physical characteristics of the nucleoprotein makes it possible that by other techniques antibody may be demonstrated. On the other hand sera from rabbits immunized with guinea pig spleen DNP affect the DNP in a manner that suggests the possibility of a specific increase in the desoxyribonuclease activity of these sera. This is being investigated further.

Effect of cortisone on pneumococcal infections in rabbits.

Effect of cortisone on experimental hypersensitivity in guinea pigs.

The production of allergic carditis, arteritis and glomerulonephritis by a single intravenous injection of crystalline bovine albumin: A quantitative study of the rate of disappearance of antigen and the development of these lesions.

Effect of cortisone on allergic carditis, arteritis and glomerulonephritis.

Effects of Cortisone and ACTH on Experimental Hypersensitivity and Circulating Antibody in Rabbits.

Purpose This project was undertaken to determine the mode of action of the adrenal hormone, cortisone, and the pituitary hormone, ACTH, in allergic disorders. Furthermore, since there are numerous pieces of evidence suggesting that hypersensitivity may play a role in initiating the rheumatic diseases, it was hoped that the solution of this project might suggest the means by which these hormones produce their beneficial therapeutic results in these illnesses.

Conclusions

The concurrent administration of cortisone to rabbits ordinarily made allergic by repeated intracutaneous injections of egg albumin markedly inhibited the development of the allergic state as indicated by failure of production of a skin response (Arthus reaction) to the injected antigen. ACTH produced a much less marked effect.

Both hormones suppressed circulating antibody and as with the Arthus reaction, the suppression produced by cortisone was much greater than that obtained with ACTH.

Both cortisone and ACTH failed to inhibit the passive hypersensitive state when antibody was supplied to the host.

These considerations strongly suggest that the inhibitory effect of cortisone and ACTH on the allergic state results from the suppression of circulating antibody.

Treatment with cortisone had no effect on the rate of disappearance of circulating antibody in the passively immunized rabbit suggesting that this hormone reduced serum antibody by inhibiting antibody formation rather than by promoting antibody destruction.

Treatment with cortisone and ACTH produced marked atrophy of the spleen, lymph nodes and thymus. The question exists as to whether this marked lymphoid atrophy is responsible for the interference with antibody production.

Whether mode of action of these hormones in the allergic disorders of man is similar to that here entertained with the rabbit is provocative. It is hoped that a similar experiment can be carried out in man.

SECTION ON EXPERIMENTAL THERAPEUTICS

Development of increased bacterial resistance to antibiotics. Considerable effort has been spent on obtaining a clear-cut answer to the question as to whether the development of increased resistance is a phenomenon of spontaneous

mutation and selection, or whether instead it represents an adaptative response on the part of the organism, with the development of new characters which persist after the removal of the drug. If the latter should prove to be the case, this will be the first instance in which the inheritance of acquired characters will have been conclusively demonstrated in bacteria. Such transformations have been described for yeast and paramecia.

Effect of ultraviolet irradiation on the development of resistance of bacteria to antibiotics. Ultraviolet irradiation of Micrococcus pyogenes var. aureus did not induce increased resistance to penicillin or streptomycin. Irradiation of culture media prior to inoculation did not induce increased resistance to penicillin or streptomycin. Irradiation of culture media prior to inoculation did not induce increased resistance to penicillin. Irradiation of Escherichia coli (K-12) caused no increase in the proportion of cells resistant to low levels of streptomycin (1-2 mg./ml.), and either no increase or a slight and evanescent increase in the proportion resistant to high concentrations (20-40 mg./ml.).

Irradiation of Escherichia coli (K-12) did, however, cause a striking increase in the proportion of cells resistant to intermediate streptomycin concentrations (4-10 mg./ml.). This reached its maximum after 11 generations, and thereafter slowly fell over a period of 50 generations without, however, returning to normal levels.

There is a strong indication that this increased resistance is not due to the mutagenic effect of irradiation on a small fraction of the bacterial population, but may instead be another example of an environmental influence which affects a major portion of the population, and which persists for a number of generations.

Role of serum albumin in supplying one or more growth-promoting factors for the Reiter treponeme. Crystallized serum albumin is a necessary component in a growth medium for the Reiter treponema which is otherwise chemically defined. Serum protein is used as a growth-promoting factor for many organisms. Davis and Dubos have suggested that the role of serum albumin is to act as a scavenger for toxic substances such as fatty acids. Others have attributed a more positive function to the protein. Our investigations show that at least one function of the commercial crystallized serum albumin used in these experiments is to supply a fatty acid to the medium in sub-toxic amounts. The lipid material in the protein can be replaced by oleic acid and several other unsaturated fatty acids. The role of the protein moiety is under current investigation.

Experimental approach to the problem of treatment failure with antibiotics. One not infrequently obtains an unsatisfactory result in the therapeutic use of an antibiotic, despite the fact that the causative organism is rapidly killed in vitro by low concentrations of the drug. A possible explanation for some of



these failures may be provided by the observation that in experimental infections in mice with group A streptococci, doses of penicillin which ordinarily effect cure in a matter of 2 to 4 hours may fail to cure an old infection even if treatment is continued for a period of 72 to 96 hours. To a lesser extent this has been found to be the case also with aureomycin and chloromycetin. This is apparently related to the fact that the organisms are not actively multiplying, and therefore not susceptible to the drug.

In collaboration with members of the staff of NIAMD, studies are now in progress to extend these observations to an experimental endocarditis of rats, produced with Streptococcus fecalis.

Further observations on the zone phenomenon in the bactericidal action of penicillin. The rate of death of certain "zone-sensitive" bacterial strains exposed to optimal concentrations of penicillin may be markedly reduced by the following addition of high concentrations of the drug. On the other hand, if these organisms are first exposed to high concentrations, and the suspension is then diluted back to the optimal level, the bacteria continue to die at the slow rate imposed by their original exposure. Penicillin at different concentration levels thus exerts two distinct effects on zone-sensitive bacteria. This suggests that penicillin may cause the accumulation of a toxic factor.

Inorganic phosphate interference with the colorimetric determination of uracil and cytosine. Inorganic phosphate interferes with the colorimetric determination of uracil with arsenotungstate, presumably due to its reaction with the latter to form phosphotungstate.

Amino acid requirements of Staphylococcus aureus (Smith) in relation to its penicillin sensitivity. A synthetic medium was developed consisting of a mixture of 12 amino acids, thiamin, nicotinamide, which gave heavy growth of the wild strain in 24 hours. Gale and co-workers have suggested that the bactericidal action of penicillin is due to the fact that the drug destroys the ability of the cell to transport glutamic acid into the cell. Contrary to that hypothesis, this strain, which did not require glutamic acid for its growth, was fully sensitive to penicillin. Microbiological assay for glutamic acid contamination of the various amino acids necessary for growth showed no significant amounts of this acid contaminating the necessary acids.

Fifteen additional mutant strains were derived with widely varying amino acid requirements, ranging from twelve acids to one. The strains requiring only one acid (either arginine, histidine or tryptophane) were ten times more resistant to penicillin than the parent strain. All strains requiring more than one acid were just as sensitive to penicillin as the parent wild type. However, on icebox storage for four weeks, most strains became at least five times more resistant than the parent strain, and the strains requiring only one acid became at least 50 times more resistant. Gale has reported similar findings, but in his experiments, increased resistance developed simultaneously with the decrease



in the amino acid requirements. No explanation can be offered at this time for the increase in penicillin-resistance of non-fastidious staphylococci on storage at icebox temperatures.

Effect of penicillin on the uracil uptake of respiring staphylococci.

In the presence of an oxidizable substrate, e.g. glucose, uracil is absorbed by resting cells. Park has reported that in the presence of penicillin, growing staphylococci accumulate an acid-labile phosphate containing uridylic acid and amino acids in undetermined configuration. In view of this observation, it was decided to test the effect of penicillin on the uracil uptake of resting staphylococci. Penicillin causes a marked increase (50 to 100 per cent) in the uptake of uracil, which may be related to Park's finding.

Mode of action of aureomycin. It has been reported (Loomis) that aureomycin uncouples the oxidation and phosphorylation of succinic acid by a liver preparation. Since this could conceivably account for the bacteriostatic properties of aureomycin, an attempt was made to repeat this observation, using cell-free bacterial enzyme preparations. Only a few cell-free bacterial extracts with phosphorylating activity have been reported. Systematic attempts to isolate such systems, using sonic vibration, autolysis in the presence of toluene, thawing and freezing, grinding with powdered glass, shaking with glass beads in the Mickle Tissue Disintegrator, and various other methods failed to yield systems capable of converting inorganic phosphorous to labile organic phosphorous. However, freshly harvested *Escherichia coli* cells ground with powdered alumina (Alcoa 301) gave a highly active cell-free preparation capable of oxidizing succinate, with a concomitant disappearance of inorganic phosphate and the appearance of labile organic phosphate.

Aureomycin in concentrations as high as 250 mg./ml. had no effect on this reaction. This is one more metabolic activity which is not the site of action of aureomycin.

Amino acid and inorganic nitrogen requirements of the Reiter treponeme.

To a basal medium containing vitamins, pyrimidines, carbohydrate and inorganic ions it proved necessary to add a number of L-amino acids for the successful cultivation of the Reiter treponeme. Of these, eleven (arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, valine) were essential, and three (aspartic acid or glutamic acid, serine, tyrosine) were beneficial. Alanine, alphaaminobutyric acid, glycine, hydroxyproline, norleucine, norvaline, and proline were inert.

Of the corresponding D-isomers none was more than one-hundredth as active as the L- form, and most were inert. None were toxic. Of the corresponding keto precursors and hydroxy analogs tested, none could be utilized. Of several glycyil peptides tested, all were equivalent to the free essential amino acid and one (glycyil tryptophan) was superior. None of the corresponding acetyl compounds were effective. Several analogs of the essential amino acids were effective anti-metabolites, one (canavanine) being very effective.

Over and above these amino acids it is necessary to add a relatively large concentration of ammonium ion. Nitrate and nitrite ions are ineffective. It would be of interest to investigate the metabolic role of the added ammonium ion, since it is unlikely that it is acting as an amino acid precursor.

The vitamin, pyrimidine, and inorganic ion requirements of the Reiter treponeme. To a basal medium containing all of the amino acids which have been found to be required for growth of the Reiter treponeme, supplemented with ammonium ion, carbohydrate, and crystallized serum albumin, it is still necessary to add several other groups of growth-promoting factors. Of the vitamins, biotin, choline, nicotinic acid, pantothenic acid, and thiamin are essential and p-aminobenzoic acid and riboflavin are beneficial. Folic acid, inositol, and the pyridoxal group are inert.

In addition to these, a nitrogenous base is required. None of the purines (adenine, guanine, hypoxanthine, xanthine) are effective, but of the pyrimidines, cytosine and uracil are equally effective as the source of the essential nutritive. Furthermore, both ferrous ion and magnesium are stimulatory and may perhaps be essential. This last point remains to be investigated in a metal-free medium.

#### SECTION ON VIRUS DISEASES

##### Minor Respiratory Viruses

Studies of Coxsackie viruses: This comparatively "new" and highly interesting group of human parasites were studied intensively from several standpoints. A highly prevalent febrile disease of childhood, herpangina, had been overlooked for almost 30 years. Community studies of illnesses occurring in households carried out in cooperation with the Epidemiology Section revealed the frequent occurrence of this specific disease entity in the Washington, D. C. area and produced evidence that this specific illness is caused by Coxsackie Group-A viruses described for the first time in 1948 but which are now known to occur as extremely common human parasites wherever they have been looked for in this country and in other parts of the world. Immunologic studies suggest that these viruses represent one of the most frequent infecting agents of man. Epidemiological and laboratory studies of two small communities, of selected populations in two hospitals and in one pediatric clinic, showed that the Group-A Coxsackie viruses do not cause illnesses similar to or identical with poliomyelitis as is popularly supposed. On the contrary, examination of over 3000 specimens for virus showed a significant association of these viruses with herpangina, a specific disease of childhood. The frequent isolations of these viruses from poliomyelitis cases and other ill persons are explained by the extremely high prevalence of this illness, by the even greater prevalence of the Coxsackie viruses, and by a marked capacity for persistence in human stools. However, implications that these viruses represent the causative agent of many such illnesses, or of any entity other than herpangina do not appear valid. It should

be stated that the findings to be reported from these studies have no bearing upon the behavior of so-called Group-B Cocksackie viruses.

The unusual value of Cocksackie viruses as tools for basic studies of the nature of small viruses, of viral behavior in laboratory animals and chick embryo culture, and as ecologic counterparts of poliomyelitis virus was realized and in part demonstrated.

The exceptional ability of the virus to survive quantitatively following unfavorable circumstances as well as specific treatment with inactivating agents led to the initiation of a number of avenues of study.

Under the direction of a visiting scientist and a biophysicist, systematic attempts were made to purify and concentrate these viruses so that they could be better characterized. This work which was performed in cooperation with a similar unit in the Virus and Rickettsial Laboratory of the Army Medical School, apparently has succeeded in achieving a degree of purity and a characterization not previously achieved with viruses of such small size (30 mu).

Because of the high resistance of crude and purified suspensions of Cocksackie to a variety of inactivating agents, including ether, alcohol, benzene, acetone and phenol and because large quantities of such viruses are excreted into human environments as well as into sewage a study was initiated to determine the effects of commonly used disinfectants upon these viruses. These studies are in part being conducted with the Environmental Sanitation Laboratory of the PHS Bureau of State Services in Cincinnati.

The successful adaptation of one strain of Cocksackie viruses to chick embryo culture in the light of failures to adapt other strains despite numerous attempts in this and other laboratories, led to studies of the pathogenesis of the adapted strain in chick embryo. These studies were performed in cooperation with the Laboratory of Pathology and Pharmacology of the NIAMD. Specific experiments were also undertaken to define if possible those factors which are of importance in adaptation of these and similar virus agents to egg culture.

#### Defense Against Bacteriological Warfare

Realization that little work and almost no progress has been evident in detection and protection against viral agents that have B.W. potential, considerable attention was given to new techniques of possible value in the collection of viruses from human environments and in their rapid recognition. This interest gave rise to a group of pilot experiments performed in conjunction with a research team at the California Institute of Technology under the direction of Dr. Alexander Goetz. These experiments were designed to test the efficiency of Molecular filters in collecting virus particles from both aerosols and hydrosols. Many problems of quantitation of virus so collected were anticipated and encountered. Investigations designed to attack these basic problems are planned for the future. Cocksackie viruses promise to be excellent tools for this purpose.



### Poliomyelitis

Attempts to adapt the non-Lansing strains of virus to small animals: By the use of a new intraspinal technic of inoculation the Leon strain has been successfully adapted to mice. Further work is being carried out to make this adaptation complete enough for practical laboratory use. Many attempts using a large variety of methods to adapt the Brunhilde strain have been unsuccessful but are being continued.

Relation of pertussis vaccine immunization and poliomyelitis: A tendency for paralysis of polio in mice to localize in the extremity where pertussis vaccine had previously been inoculated has been demonstrated. This tendency is not marked and required large numbers of animals to substantiate. Future work on this project will attempt to evaluate the effectiveness of other types of inocula in producing this localization of paralysis and the time relationships necessary.

The effect of nasal mucus on susceptibility to polio:- Preliminary studies show a relationship between relative humidity and epidemic spread of polio. These must be substantiated by a larger experience in different populations.

Propagation of virus in tissue culture: Lansing strain of virus has been grown in monkey testicle and kidney tissue. It is planned to use this tissue culture technic of polio virus growth in the fundamental studies of virus-cell relationships.

Attempts to adapt the Lansing strain to chick embryos have been unsuccessful and will not be pursued further at this time.

Antiviral substances from brain: A phospholipid fraction of normal beef brain has been chemically isolated which neutralizes in vitro most neurotropic viruses but has no effect on influenza. Its effect appears to be directly on virus and is not demonstrable in vivo.

### Common Cold

The work on this project during fiscal year 1952 will be incorporated into that of the Unit on Minor Respiratory Viruses.

Repeated attempts during fiscal year 1951 to produce consistent clinical infection in human volunteers with chick embryo passage of MR-1 virus have been negative. This virus, therefore, is no longer available for future work. Evaluation of MR-3 materials is continuing with no conclusions possible at the present time.

### Infectious Hepatitis

This project was initiated during the fiscal year 1951 and replaces the project on infectious mononucleosis. The approach has been chiefly that of



attempting to cultivate the virus of infectious hepatitis in tissue culture and the developing chick embryo. As a necessary corollary an investigation of methods of demonstrating the presence of hepatitis virus has been initiated through the use of cultivated inactivated virus as a skin test antigen. From preliminary results it would appear that the virus has probably been cultivated in chick embryos which confirms the work of Henle et al, although the limitations of the skin test in human cases has yet to be worked out. The need of human volunteers as a means of demonstrating the presence and nature of this virus has been recognized and in collaboration with the Laboratory of Biologics Control's program on serum jaundice steps are being taken to establish such a volunteer program.

#### Newcastle Disease Virus

Limited basic studies of virus infection using Newcastle disease virus as a prototype have been completed. It has been shown that this virus can be adapted to the suckling mouse by intracerebral inoculation causing a fatal encephalitis. However, of two strains thus adapted only one has been successfully transferred from suckling to adult mice. The second caused death of adult mice but could not be maintained in serial passage probably representing an incomplete growth cycle of the virus.

Electron microscope studies on chick embryo tissues infected with Newcastle disease virus have been completed in collaboration with the Laboratory of Physical Biology, National Institute of Arthritis and Metabolic Diseases has been demonstrated in relation to cell structure.

It has not been possible to carry out a proposed study of mouse adapted virus in the vaccination of poultry, but the virus has been made available to agricultural research workers.

### SECTION ON BACTERIAL AND MYCOTIC DISEASES

#### Brucellosis Unit

Metabolic, physiological, epidemiological, and clinical studies of the brucella have been continued.

Using the Warburg technique a combination of aureomycin and dihydrostreptomycin was found to act synergistically in inhibiting the respiration of brucella. This finding helps to explain the clinical effectiveness of this combination of antibiotics in the treatment of brucellosis.

Further human cases of brucellosis have been treated with a combination of three drugs: a sulfonamide (sulfadiazine or triple sulfonamide), Aureomycin, and Dihydrostreptomycin. The results have continued to be excellent. This treatment appears to be the most effective used to date in this disease.

The studies on experimental human salmonellosis conducted at the University of Chicago have been concluded. Twelve strains of Salmonella, derived from spray-dried whole egg, representing six species, Salmonella meleagridis, S. anatum, S. newport, S. bareilly, S. derby, and S. pullorum were fed to human volunteers under controlled conditions. Over four hundred individual feedings were conducted. All of the strains fed proved pathogenic for man. Many asymptomatic carrier states were observed and studied as well as nearly 100 cases of clinical illness. The results of this work should settle the controversy in regard to the public health importance of Salmonella species in market samples of spray-dried whole egg.

### Mycotic Diseases

Study of fungicides:- In vitro screening of fungicides is being continued on a limited scale. In vivo testing of fungicides against histoplasmosis has yielded several agents of some promise.

Studies of naturally acquired histoplasmosis in trapped animals is being continued.

Search for pathogenic fungi in soil is being continued.

A study of an outbreak of cryptococcus infection in cattle has been carried out with repeated isolation of the organism from milk.

An apparently new species of fungus (Cladysporium cerebrale) has been isolated from diagnostic materials submitted from two patients.

### Rheumatic Fever

An attempted evaluation of the role of streptococcal albuminates in rheumatic fever has continued with no definitive results. This phase of the work will probably be concluded in the coming year. At that time a field study of the epidemiology of streptococcal infections and rheumatic fever will be initiated. The purpose of this study will be to determine the factors involved in the geographical distribution of these diseases in civilian populations.

### Tuberculosis Unit

#### Vaccine Studies

Studies of BCG, both as a liquid suspension and as a dry vaccine have been continued with particular emphasis on methods of counting viable particles. Excellent growth in liquid medium has been obtained, resulting in a more satisfactory counting technique. Studies of a virulent strain of Mycobacterium tuberculosis killed by ultra-violet light as a potential vaccine, will be continued during the coming year. Fractionation of tubercle bacilli with studies of the

antigenic properties of these fractions continues. A cooperative study has been started to examine the effect on tubercle bacilli of a cathode ray beam. A repetition and expansion of work by Vorwald et al has been initiated to study the effects of the combinations of BCG, silicosis, and alterations of the pulmonary vascular system in guinea pigs and monkeys.

#### Chemotherapeutic Studies

Approximately 1000 newly synthesized drugs have been tested in vitro for activity against a saprophytic specie of Mycobacterium, with those drugs showing promise being extensively tested in vivo.

#### Basic Studies in Tuberculosis

The cooperative study of the effect of alteration of the pulmonary vascular system on the evolution of pulmonary tuberculosis in monkeys continues. The previous results of anastomosing a systemic and pulmonary artery have been substantiated by additional experiments introducing other variable factors.

#### Field Studies

Long range study of the etiology of pulmonary calcification is being conducted in Loudoun County, Va., and in Alabama, in conjunction with supportive laboratory studies.

#### The Evaluation of a Blood Cell Counting Instrument

In conjunction with an NIH Bio-phycisist, of the National Institute of Arthritis and Metabolic Diseases, a newly developed instrument for the determination of red blood-cell and white blood-cell counts and hemoglobin determination based upon photoelectric methods, has been evaluated. Studies of methods of counting of suspended particles based on similar principles will be undertaken in the coming year.

#### Diarrheal Disease Investigations

Investigations of the diarrheal diseases were carried on in F.Y. 1951 in New Orleans, Louisiana, Thomasville, Georgia and Bethesda, Maryland. The first two study areas are jointly sponsored with the Communicable Disease Center of the Bureau of State Services.

In Bethesda, progress has been made in the typing of members of the Pseudomonas group of organisms, thus permitting a more definitive investigation of the relation of these organisms to human disease. \* Typing sera for members of the Proteus group were also prepared.

In Thomasville, epidemiological studies were continued in relation to fly control. It has now been shown that fly control was of value in reduction of

Shigellosis in this area. The acquired resistance of flies to various insecticides has shifted the emphasis of the studies to fly ecology and to an investigation of the natural sources of Salmonellosis.

New Orleans studies in a newborn nursery have continued. An outbreak of Salmonellosis in this nursery, still in progress, is resulting in valuable information on the actual mechanisms of spread in the nursery. To date some twenty-five infections have occurred in the nursery and numerous recoveries of the organism have been made from the inanimate objects of the environment. It appears certain that useful improvements of nursery technique can be based on these findings.

A co-operative study in California (Fresno County, State Health Department, Communicable Disease Center and National Institutes of Health) was initiated in 1951 and field work was carried on for six months. It was shown that a major cause of diarrheal diseases in farm workers was Shigellosis. Control measures based on this knowledge have been recommended.

At the request of the Brazilian Government and the Institute of Inter-American Affairs, a survey of the diarrheal disease problem in the Amazon Valley was made. Proposals for study were accepted and are now under way. Consultation and other assistance are continuing.

#### SECTION ON EPIDEMIOLOGICAL STUDIES

##### Field Studies

Brucellosis	Immunity mechanism
Calcified pulmonary lesions	Pertussis Immunization
Diphtheria immunization	Rodent octoparasites
Histoplasmosis	Salmonella (epidemic)
Influenza	fever

The field data for the studies on brucellosis, rodent octoparasites, Salmonella, immunity mechanism, aureomycin treatment of pertussis, and Q fever have been collected and await analysis evaluation and report.

The field data on other studies is still in the process of collection.

Field studies on sarcoidosis, Coxsackie Group-A infections, and conjunctivitis were concluded.

Sarcoidosis - cases of sarcoidosis which occurred in Army personnel during World War II have a peculiar geographic distribution with a high prevalence among persons born in the Southern States particularly the South Atlantic Region.

Group A Coxsackie Viruses - observation on the natural occurrence of Group A Coxsackie viruses in a limited population group showed that these



infections have characteristics similar to common communicable disease patterns. They have a very distinct seasonal distribution. Their spread apparently depends upon contact infection and the younger age groups are more prone to be infected. All epidemiological and immunological data indicate that at least some of these viruses are responsible for one communicable disease observed, namely Herpangina.

Influenza - The study of an acute epidemic among children residing in an institution showed that the cause of the epidemic was influenza and the epidemic pattern was consistent with that disease.

Aureomycin treatment of pertussis - More than 100 cases of pertussis have been treated in the home with aureomycin. Although the data have not yet been completely analyzed, it is obvious that such treatment is practical and specifically benefits the course of the disease. From the Public Health standpoint, 80 to 90% of all cases had positive cultures before the onset of treatment and only 20% had positive cultures after 3-4 days of treatment. This is important in limiting the spread of infection.

Q Fever - The results of some of the epidemiological and epizootological studies of Q fever in Los Angeles were summarized with the practical conclusion that although effective pasteurization of milk would help to prevent some infections and vaccination of occupational exposed groups may afford protection to those groups, it appears that truly effective control of the disease in man must await development of measures for control of infection in animal sources of human infection.

Conjunctivitis - Bacteriological and field studies begun last year in Thomas County, Georgia on acute conjunctivitis were continued through the year and showed that the principal organism isolated from acute cases was Hemophilus aegyptius (Koch-Weeks bacillus). The disease is most prevalent in children and occurs during the summer and early fall. In a study in elementary schools, the disease was found to be an important cause of school absenteeism. This project has been concluded and the data will be analyzed during the coming year.

Other studies - Careful evaluation of reported association between paralytic poliomyelitis and injections with vaccines, penicillin and other substances.

As Chairman of the U. S. Delegation, the Section Chief collaborated with delegates from all World Health Organization member states in preparation of International Sanitary Regulations which were adopted by the 4th WHO Assembly in Geneva.

Regional Influenza Study Program - The Influenza Information Center of the World Health Organization Influenza Study Program in the United States has been located in this laboratory during the past year and reports from the various

laboratories participating has been summarized weekly for publication in the Communicable Disease Summary of the National Office of Vital Statistics. These reports indicated that there was an unusual prevalence of influenza throughout the U. S. during late January, February and early March, and that it was chiefly due to an A' type of virus closely resembling that isolated the previous year. In general the disease was mild with few deaths and there was no evidence of increased fatality in the young adult age group. Laboratories participating in the Influenza Study Program reported 79 isolations of A' influenza virus, 2 type A, 1 type B, and 1 type C from clinical cases. A total of 958 paired sera have shown significant rises in antibody titer for the general type A, and 22 paired sera have shown rises for type B influenza virus. Under the auspices of the Influenza Study Program, licensed manufacturers of influenza vaccine were requested to prepare as quickly as possible 1,000 doses of monovalent vaccine using a strain recently isolated in London. Three manufacturers accomplished this in less than 24 days, and 3 others in a slightly longer time. This was done to give a basis for an estimate of the time required to produce larger amounts on an emergency basis.

Sectional Research Program for Biological Warfare Defense. In order to encourage original research by independent investigators outside of the National Institutes of Health on diseases which may be important in biological warfare defense, and to expand and strengthen existing civilian laboratory facilities for the specific diagnosis of outbreaks of diseases not ordinarily included in routine laboratory techniques, plans have been developed for a Sectional Research Program for Biological Warfare Defense, based in part upon the experience of The Influenza Study Program. Coordinating Laboratories have been selected in each of 12 geographic regions of the United States to be assisted by other collaborating laboratories selected from State Health Departments, universities, research institutions, and hospitals. Funds for the organization and to support research projects will be administered through the established research grant program of the National Institutes of Health. (See page - under Extramural Programs Branch)

LABORATORY OF TROPICAL DISEASES  
Willard H. Wright, Chief

The broad field of tropical medicine is the primary research interest of this Laboratory. Employing both field and basic laboratory approaches, investigators seek improvement in diagnosis, treatment, and control or cure of parasitic diseases important not only domestically and militarily, but also in terms of their importance to trade with tropical areas and the economic welfare. This Laboratory's research approach varies from the fundamental - such as studies on physiology and host-parasite relationships - to such work as chemotherapy to develop better treatment for such diseases as amoebiasis and human schistosomiasis. Specific projects and studies are discussed below in summary form, together with the more important findings of the past fiscal year.

Studies on the diagnosis, pathology, symptomatology, and host-parasite relations of the helminths which infect man were continued with projects on:

Biology of the schistosomes

Identification, distribution, and ecology of the vectors of schistosomiasis

Comparative morphology of the skin-inhabiting microfilariae of man and domestic animals

Development of Onchocerca volvulus in Simulium

Metabolism of nematode larvae cultivated in vitro

Factors influencing the development of microfilariae in mosquitoes

Biology of Wuchereria bancrofti occurring in Tahiti

In connection with studies on the methods of preventing human infection with pathogenic parasites, work continued on:

Follow-up examinations of treated onchocerciasis patients

Screening of compounds for molluscicidal activity with field testing of the more promising ones in West Africa and Brazil

Genetic influences on susceptibility of snails to infection with schistosomes

Testing of compounds for cysticidal activity against Endamoeba histolytica

Evaluation of various control measures against filariasis in Tahiti

Research on the biology, ecology, physiology, and taxonomy of arthropods of medical importance was continued with projects on:

Determination of possible arthropod vectors of toxoplasmosis

Developmental morphology and physiology of the mosquito

Physiological and morphological changes produced in arthropods by poisons

Diagnostic characteristics, distribution, ecology, and biology of the Simulium vectors of onchocerciasis in Guatemala

Screening of compounds for larvicidal activity against Simulium

Relationship of *Culicoides* to transmission of onchocerciasis

Identification, ecology, and distribution of vectors of filariasis in Tahiti

Developmental characteristics of larval forms of filariae as found in the mosquito

Influence of microfilariae counts and antifilarial therapy on infectivity for mosquitoes

In an attempt to evaluate the factors influencing the epidemiology of tropical diseases, projects were continued on:

Cytology of trypanosomes and Plasmodia

Effect of various therapeutic agents on transmission of malaria

Biology of Plasmodia

Factors influencing the spread between individuals of intestinal parasites (using human volunteers)

Incidence of parasitic infections and related environmental factors in a rural community of 500 families

Comparative efficiency of various diagnostic methods for Endamoeba histolytica

Possible role of flies in transmission of intestinal protozoa

Prevalence of human toxoplasmosis as indicated by the dye test and by recovery of organisms from anti- and post-mortem material

Status of domestic animals and rats as possible reservoir hosts of Toxoplasma gondii

In connection with investigations on the morphology and biology of the pathogenic protozoa of man, projects were continued on:

Growth requirements and factors influencing excystation of Endamoeba coli and Giardia lamblia

Metabolism of E. coli, E. histolytica, and Dientamoeba fragilis

Enzyme systems of the trypanosomes



Development of drug resistant strains of trypanosomes

Evaluation and improvement of serological dye test for toxoplasmosis

Virulence and invasiveness of Toxoplasma gondii

In the course of studies on the mode of drug action and development of new and more effective drugs for treatment of parasitic diseases, projects were continued on:

Course of Plasmodium berghei infection in rodents

Attempts in transmission of P. berghei

Application and identification of metabolites of pamaquine

Testing of drugs for antimalarial activity

Testing of drugs for activity against E. histolytica

Effect of chemotherapeutic agents on intestinal helminthiasis in man, on toxoplasmosis in mice, and on schistosomiasis in mice

Clinical evaluation of the oxyquinolines in the treatment of malaria induced in human volunteers

Studies on the basic physiological processes of pathogenic parasites and their intermediate hosts and vectors were continued with projects on:

Influence of trypanosomes on the respiratory metabolism of rats

Influence of ACTH on gluconeogenesis in trypanosome-infected rats

Intermediate metabolism of anaerobically kept snails

Mechanism of aerobic respiration of snails

Mechanism of drug resistance of trypanosomes

Metabolism of the larvae of Trichinella spiralis

In clinical immunology, projects were continued on:

Mechanism of immunity

Application of the principles of immunology to the study of biological activity of parasites

Development of better methods and more specific and potent antigens for the diagnosis of parasitic diseases

Major findings:

Large percentage of Aedes pseudoscutellaris will become infected after feeding on filariasis patients with low mf. counts which were previously thought to be non-infective.

Mammalian toxicity studies with several of the more promising molluscacides show them to possess a satisfactory margin of safety.

A quantitative test for microgram quantities of certain molluscacides in natural waters has been developed. A similar quantitative test has been developed for determining the amount of the chemical in the tissue of snails exposed to the compounds.

Results obtained from screening of over 300 compounds containing a benzene nucleus with an attached OH group permit the formulation of some definite theories concerning molluscacidal activity.

Individual flies fed on  $P^{32}$  are easily identified even one month after feeding.

Human volunteers have been successfully infected with 10 and 100 cysts of Giardia lamblia, the prepatent period being 8 to 11 days. Infection with Endamoeba coli was produced in one volunteer eight days after ingesting one cyst while others have been infected with 100 cysts. Clinical symptoms were not noted.

It is indicated that patients with vivax malaria may serve as a source of infection to mosquitoes before the disease can be diagnosed.

Although chloroquine alone is a good therapeutic agent against malaria, it does not prevent the infection of mosquitoes for several weeks following treatment due to its failure to act on the gametocytes.

Toxoplasmas persist in pigeons for at least one year following inoculation. This is the only animal so far found to exhibit a high parasitemia in the absence of acute disease.

A microculture method was developed for the in vitro testing of drugs for their activity against E. histolytica.

From microisolated cysts, E. coli has been cultivated with known species bacteria.

Cysts of E. coli have been found to withstand prolonged drying in contrast to those of E. histolytica.

Toxoplasmosis was apparently experimentally transmitted by Dermacentor variabilis (1 animal out of 17 becoming positive) and by D. andersoni (2 animals out of 58 showing infection).

Two new compounds showed considerable promise as effective agents against amoebiasis in the guinea pig.

Various carbohydrates fed in a synthetic diet have been found to either enhance or suppress experimental amoebic infection in rats.

All attempts at insect transmission of Plasmodium berghei have failed.

Four metabolites of pamaquine have been isolated and one identified.

In trypanosome-infected rats, the increase in oxygen consumption has been found too small to allow the assumption that the organism kills its host by asphyxiation.

A new, rapid, and sensitive flocculation test has been developed which gives promise of having wide application in the field of sero-diagnosis.

#### Projects concluded during the year:

Studies on the arthropod transmission of toxoplasmosis.

A study to determine the incidence of pinworm infections among a group of patients with poliomyelitis.

LABORATORY OF BIOLOGICS CONTROL  
William G. Workman, Chief

The Laboratory of Biologics Control is concerned primarily and directly with the administration of the Biologics Law (Public Law 410- as amended) which is directed at assuring the public of safe, potent and sterile biological products such as serums, toxins, antitoxins, human blood and blood derivatives.

The control of biological products in interstate commerce and in the export and import trade is achieved by a process of licensing in which manufacturing establishments, as well as the specific products involved, are licensed. Licensing is contingent upon the manufacturer meeting and maintaining certain standards which is insured by periodic inspection of establishments and continuous check of manufactured biological products. The latter is achieved by requiring submission of samples and testing records to this laboratory for control testing and inspection. In addition the Minimum Requirements for each

product are revised from time to time to take notice of scientific advances in the field. Standard biological preparations are issued for the use of those concerned with the manufacture of biological products. Rapport with personnel of licensed laboratories continues to be excellent as it has been in the past and control is achieved by a process of cooperation rather than of policing.

During the past year the technical and administrative resources of this Laboratory were placed under a severe strain because of additional responsibilities arising mainly as a result of the expansion of the National Blood Program. This involved the licensing of additional establishments and inspection of Red Cross Regional and Defense Blood Centers to enable them to cooperate with the program. The impact of these activities is reflected in the figures appearing later in this report.

The program of work in the past year included the following:

Control of biologics: At the close of the Fiscal Year 1951 a total of 112 establishments were under license with 227 separate products being licensed making a total of 1302 individual product licenses. Establishments in the United States, Canada, Mexico, Puerto Rico and Hawaii were inspected during the year. Methods of production and testing were reviewed with the responsible technical personnel in each laboratory and suggestions made for improving methods where necessary. In all a total of 138 establishments and their subsidiaries were inspected. These included 26 new blood banks and 43 Red Cross Regional and Defense Blood Centers.

Minimum Requirements for Licensed Products: New or revised Minimum Requirements pertaining to safety, purity and potency were issued for the following products:

Normal Human Plasma  
Smallpox Vaccine  
Typhoid Vaccine

In addition tentative Minimum Requirements were issued for:

Antirabies Serum  
Antiserum for the Agglutination-Inhibition Test for Influenza Virus (Dried)  
Influenza Virus Hemagglutinating Antigen, Chick Embryo Type (Dried)  
Streptokinase-Streptodornase

Control tests of Licensed Products: Control tests of biological products submitted for release or obtained at the time of annual inspection are shown below:

Samples Submitted for Release

4,533\*

\*Includes 1,271 samples of Normal Human Plasma



Samples received following annual inspection	658
Total	<u>5,191</u>
Tests for Potency	1,891
" " Sterility	4,124
" " Pyrogenicity	1,463
" " Toxicity	12
" " Stability	41
Moisture determinations	61
Hemoglobin determinations	452
Misc. tests	147
Total Tests	<u>8,191</u>

A total of 2,265 lots of products were released on the basis of the Manufacturers Protocols and 1,835 following test in this Laboratory.

Biologics Standards: Manufacturers of biological products and institutions working in the field were supplied with standards and cultures as follows:

Biological Standards	1,755
Bacterial Cultures	400
Virus preparations	90

Miscellaneous Tests:

Cultures for identification	15
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Plasma Testing Program: Although actually a part of the general program of Biologics Control the plasma testing program should be mentioned separately because of the magnitude of the operation, most of which took place during the last 5 months of the fiscal year. Because of the small number of units which constitute a lot of plasma it was considered practical to examine and release plasma lot by lot only in the case of new manufacturers. Once an establishment demonstrated its ability to manufacture satisfactory plasma two units were required for test purposes for each 1,000 manufactured. Plasma was tested for sterility and pyrogenicity in all cases, the test for sterility being conducted in such a manner that the auxilliary apparatus designed for reconstitution and administration of the plasma was also tested. In addition tests for hemoglobin, protein and moisture content are carried out. If all the tests performed were satisfactory a second sample from the same lot was made available for administration to a hospital patient in need of plasma. Releases were based on a review of the manufactures protocol (showing details of processing including ultra-violet irradiation) and the tests performed in this Laboratory as well as on the results of clinical administration. The figures below indicate the scope of the work performed during the fiscal year.

Total number of lots of plasma submitted for release	1,271
Pyrogen tests	1,463
Hemoglobin determinations	452
Units provided for clinical administration	580
Clinical reports received	375

Blood and blood products: A great deal of effort was put into the solution of problems encountered in the irradiation of plasma in order to improve the safety and quality of irradiated plasma. The majority of the activities described below deal with such problems, arising particularly in the Dill ultraviolet irradiator, a commercially produced modification of the irradiator described by Habel and Sockrider. The basic data are nevertheless applicable to other types of ultraviolet irradiators. The following projects were successfully completed during the past year.

(a) A micro-irradiating chamber was developed for use with the Dill ultraviolet irradiator. With this device it is possible to irradiate from 1.0 to 10 ml. of fluid under conditions similar to those encountered when processing at rates of 100 to 500 ml. per minute.

(b) A simple method of continuously measuring a constant sample of incident ultraviolet light in an irradiator was developed. This was done to demonstrate the practicability of building a continuous recording meter. This has not been written for publication but the information has been used by Mr. Hugh M. Archer, an engineer, working under a Research Grant from the Public Health Service in the construction of a laboratory model recording-controlling monitor suitable for controlling the irradiation of plasma.

(c) A laboratory model of the above monitor for plasma irradiation was tested and specifications developed for a further monitoring device suitable for commercial irradiation of plasma.

(d) In cooperation with the instrument shop of the National Institutes of Health a pump for delivering a constant and steady flow of plasma within a sterile closed system has been developed. The use of this pump will greatly simplify processing of plasma and when used with a controlling recorder will permit simultaneous irradiation and filling into final containers and thus reduce the time of processing.

Study is being pursued along the following lines in order better to define the conditions of irradiation in irradiators consistent with maximal desirable effect on the liquids being irradiated.

(a) To secure basic information preliminary to the development of a device that will control the irradiation of plasma by continuous monitoring of the amount of ultraviolet light absorbed.

(b) To study the effects of temperature on incident energy, absorbed energy, film thickness and biological effectiveness in the irradiation process.

(c) To determine by photographic methods film thickness and exposure time at various flow rates.

(d) To study the effects of varied current inputs on the output of ultraviolet light and the temperature of the irradiator chamber.

(e) To study the phenomenon of photoreactivation of microorganisms inactivated by ultraviolet light, under controlled conditions of irradiation.

(f) To investigate the effects of different levels of irradiation on plasma components.

(g) To compare the effects of common anticoagulants on the components of whole blood and methods of blood collection.

(h) To test a modified irradiation system with controlled air flow and to determine the effect of different gases on chamber and lamp temperatures, incident energy, biological effectiveness of irradiation, and on the plasma components.

(i) To study the effects of lipids on ultraviolet transmission by plasma.

(j) To determine the maximum speed of processing obtainable with the existing irradiation apparatus and the minimum length of irradiation chamber necessary.

(k) To investigate the use of radioactive isotopes for sterilizing whole blood.

Hepatitis studies: Dr. J. W. Oliphant reported for duty on March 5, 1951, to engage in a comprehensive study on infectious hepatitis, with particular reference to its relationship to the National Blood Program, in cooperation with the Laboratory of Infectious Diseases. Arrangements have been made with the Federal Bureau of Prisons to do inoculation studies in human volunteers from among the inmates of the Lewisburg, Pennsylvania Federal Prison and the Federal Correctional Institution at Ashland, Kentucky and to do a large scale study of a specific skin test for infectious hepatitis at the Federal Penitentiary at Leavenworth, Kansas. This latter study will be done in cooperation with Dr. Lawrence Kilham of the Laboratory of Infectious Diseases. Arrangements have been made to prepare a large pool of Normal Human Plasma to which will be added serum from acute phase cases of serum hepatitis. This pool is planned to serve as a large volume of material of known infectivity for studies to be performed on the standardization of ultraviolet irradiation as an agent for the inactivation of hepatitis virus and for testing any other promising physical or chemical inactivating

agents as well as for other studies on the virus, including studies of the infectivity of various fractions of human plasma, which may develop.

Experimental syphilis in rabbits: Studies of resistant strains of *Treponema pallidum* were continued. The Nichols strains of *T. pallidum* in experimental syphilis was not rendered penicillin-resistant by large single subcurative treatments in three consecutive rabbit passages. It has been necessary to curtail the scale of this project because of the calls made upon the time of personnel by the plasma testing program.

Rabies virus and vaccine studies: The study of the factors in rabies vaccine and normal rabbit brain tissue which cause neurological symptoms (allergic encephalitis) in guinea pigs has been continued and progress is being made in the development of a simple procedure for purifying rabies vaccine.

Studies on a graded dose method of testing the antigenicity of rabies vaccine have been continued. A two-dose method is under scrutiny and to date seems entirely satisfactory.

A tentative reference Rabies Vaccine (dried) has been prepared for use by licensed laboratories. From experience gained with the use of this vaccine it should be possible to prepare a reference vaccine which will facilitate the standardization of Rabies Vaccine produced in licensed laboratories.

Histamine sensitization of mice induced by pertussis vaccine and Hemophilus pertussis. These studies follow those reported last year under Encephalopathy produced by Pertussis Vaccine. The findings reported below are of particular interest in that they may yield clues as to the nature of pertussis in the child.

Findings: Mice are relatively insensitive to histamine, but as was previously reported pertussis vaccine may increase the sensitivity 50 to 100 fold. The female mouse, both normal and vaccinated, is more sensitive than the male. The female mouse is also better protected by the vaccine. Vaccines providing the best protection induce the greatest degree of sensitivity. Mice infected by the intranasal route develop a respiratory infection with low mortality. After an incubation period these animals develop a high degree of sensitivity which is related to the presence of lung lesions which may last up to 3 weeks and from which the specific bacteria may be recovered. Sensitization may last for more than 50 days.

Antipertussis serum. Work has been renewed on the development of a potency test for this product. Some complicating factors have been evaluated and it is anticipated that it will be possible to issue Minimum Requirements for this product.



Influenza virus and vaccine. The study of Influenza Virus strains from epidemics and outbreaks in many parts of the world and occurring at different times have been continued in an effort to determine the effectiveness of present influenza virus vaccine and to improve them. Particular attention has been given to strains against which the commercial influenza virus vaccine are ineffective. Representative strains have been sent to licensed manufacturers so that production of vaccines from such strains could be started quickly should outbreaks due to similar strains occur.

A new Reference Influenza Virus Vaccine containing four component virus strains was obtained, standardized, and distributed to licensed manufacturers.

Virus strains from the serious influenza epidemics in England and the continent of Europe in January 1951 were similar antigenically to two of the strains used in commercial vaccines manufactured in the United States of America. This was not known at the time and a strain from the epidemic in England was quickly obtained, cultivated in embryonated eggs and sent to commercial laboratories who undertook to make trial lots of vaccine. One firm completed manufacture of the vaccine in 3 weeks while all 6 had completed the work within 57 days. This is significant since it gives some information as to the minimum time required to produce a finished vaccine, once having obtained the strain.

An investigation of the drying of Influenza virus vaccine is being undertaken. Further stability tests are necessary before conclusions can be drawn.

Studies of Influenza virus in the cells of embryonic chick lungs are being continued with Dr. Wycoff.

#### Investigation of the Secondary toxins formed by Diphtheria Bacilli.

Findings: A "Standard chick" has been experimentally defined, the minimum Lethal dose and L / dose of the regular toxin used in this Laboratory has been determined for such chicks in terms of the Standard Diphtheria Antitoxin.

Relationships among Meningococci. Clarification of relationship among meningococci with the purpose of selecting reliable strains of the various serological groups to be used for reference and as antigens for preparation of specific diagnostic antisera was undertaken in response to numerous inquiries concerning a solution of a significant epidemiological problem.

Findings: So far the study chiefly concerns the Group II alpha and the numerous strains in the laboratory collection have been screened by agglutination. More intensive study of the most promising strain has been begun.

Dysentery toxin: Studies of the toxins of Shigella dysenteriae (Shiga) have been continued along the following lines.

(a) The fundamental nature of these toxins is being studied.

Findings: Shiga toxin is non-dialysable although much of the associated nitrogen is. The toxin is precipitated at  $pH$  4.0.

(b) Development of toxoid from Shiga toxin. The toxin is converted into toxoid by ultraviolet irradiation, by heat and by formaldehyde. Toxoids prepared by these means were compared for antigenicity, as plain and alum precipitated antigens, in mice, using varying conditions of dosage, time of immunization and challenge.

Findings: The toxoid obtained by careful heating at a definite temperature for a definite time was of high antigenicity, more stable and more easily reproduced than those obtained by ultraviolet irradiation or by the use of formaldehyde.

(c) The phase distribution of Shigella sonnei in cases of dysentery was studied with a view to determining how valuable an antigen prepared from S. sonnei would be in vaccinating against dysentery.

Findings: In cultures from rectal swabs of 100 acute cases of dysentery due to S. sonnei 80-95 per cent of the colonies have been found to be of Phase 1.

The role of Shiga toxin in the intestinal tract of monkeys (Macacus mulatta) has been concluded. Certain phases of the work have been reported before the Society of American Bacteriologists.

Findings: Shiga toxin produces no symptoms in monkeys when introduced into the intestinal tract by mouth, or when placed repeatedly in isolated pouches of the intestine. It is readily absorbed, however, and produced a high titer of antitoxin in the blood of those animals which are then immune to intravenous toxin in doses which are constantly fatal to untreated monkeys. Furthermore, their blood protects mice against the toxin.

Effects of cortisone on Shiga intoxication and on meningococcus infection in mice. In last years' report a study of Cortisone and its effect on diphtheria intoxication in guinea pigs was reported. During the past year the studies were extended to include its effect upon Shiga toxin and on meningococcus infection in mice with and without ascorbic acid.

Findings: Both Shiga toxin and Meningococcus cultures caused marked hyperemia and an engorgement of the adrenals in mice. Cortisone did not prolong the life of these animals but it did have a pronounced sparing action upon the adrenals, relatively few cortisone treated mice showing red adrenals.

Smallpox control vaccine (dried). Preparation and standardization of this material was successfully concluded and it is now available to licensed manufacturers for control purposes.

Project discontinued. The proposed "Pertussis Vaccine Field Project" to be initiated in 1950-51 in cooperation with Dr. J. A. Bell, Laboratory of Infectious Diseases, was abandoned because of the world situation.

ROCKY MOUNTAIN LABORATORY  
Carl L. Larson, Chief

The Rocky Mountain Laboratory continues to emphasize a basic research approach to studies on virus and rickettsial diseases of particular importance to public health in the Western United States. In recent months, this Laboratory has been able to round out its research program to include work in such fields as biophysics and western diarrheal diseases and to strengthen its biochemical studies to include radioactive techniques. Specific research programs will be discussed below along with the more important accomplishments of the past fiscal year and with program plans for the future.

#### Rickettsial infections

Attempts are being made to adapt strains of certain rickettsiae to infect and to survive in mice. Previous observations in the literature indicate that epidemic typhus fever rickettsiae are unable to survive serial transfers through mice, while murine typhus fever rickettsiae can survive an indefinite number of such passages, although there is considerable variation in the lethal effect noted. In this laboratory the epidemic strain has been readily transferred through a series of mice, and the murine strain has consistently killed approximately 100 percent of the animals through which it was passaged. Studies to investigate some of the factors that might influence adaptation of rickettsiae to survival in mice indicate that animals receiving minimal immunizing doses of either strain of rickettsiae develop homologous immunity in less than 5 days. In conjunction with this observation it was found that when larger doses of rickettsiae originating from epidemic typhus fever are inoculated into mice, maximum growth occurs on the second day, a sudden drop of recoverable rickettsiae occurs on the third day, and minimal numbers persist up to the seventh day after infection.

Further studies to determine whether or not the so-called "toxin test" is of greater value than the intraperitoneal-infection test in determining the presence or absence of inapparent infections in mice indicate that the "toxin test" does not detect inapparent infections before the thirtieth day after administration of the first infective dose, whereas the intraperitoneal-infection test is usable as early as the tenth day after the original infection.

Q fever vaccine project. Q fever vaccines are being evaluated with a view to applying the results to field studies among infected herds of cattle in



California. Various methods are being employed to produce antigenic materials, and the factors necessary to evaluate the potency of these preparations are being analyzed. It is apparent from the literature that very little is known in a comparative way about the potency of vaccine preparations or about methods to evaluate these vaccines. Thus, the fundamental immunological aspects of Q fever are being investigated in this project. Disintegration of Coxiella burnetii produces suspensions which eliminate the property of producing severe local reactions when injected into cattle, but likewise reduces to some extent their antibody-stimulating activity in guinea pigs. These suspensions of disintegrated rickettsiae, however, do confer considerable protection upon guinea pigs and if they continue to prove to be effective immunizing agents, two of the principal limitations of fever vaccines for use in large-scale vaccination of cattle may be removed. These limitations are the production of severe local reactions and the production of detectable complement-fixing antibodies in cattle that have been vaccinated subcutaneously.

With regard to production of Q fever vaccines, an investigation is now under way to determine the relationship between the potency of Q fever vaccine and the complement-fixing value of such vaccines. It has been shown that the number of infectious doses per complement-fixing unit in terms of the chick embryo and the guinea pig are  $1.3 \times 10^5$  and  $1.3 \times 10^6$  for embryos and guinea pigs, respectively. This suggests that the complement-fixing unit may be employed as a measure of the number of organisms present in a preparation and also that the complement-fixing value may be related to the potency of vaccines prepared from rickettsiae. It is possible from the data thus far assembled to select with some degree of accuracy the period at which maximum numbers of rickettsiae may be harvested from chick embryos, which is obviously a factor of importance in the production of vaccine.

A manuscript has been prepared recording the results of a pilot study to test the use of vaccines in the control and prevention of Q fever in dairy herds in California. The data were assembled on the basis of the complement-fixation reaction and on the isolation of C. burnetii from milk. Three times as many nonvaccinated as vaccinated cows gave serological evidence of infection, and five times as many nonvaccinated as vaccinated cows were shedding C. burnetii in their milk. Since the greatest number of infections occurred soon after the animals were placed in infected environments, it would appear that repeated vaccination of individual cows might not be necessary to control the spread of Q fever in a herd.

During the spring of 1951, two cases of Q fever, diagnosed on the basis of an increasing antibody titer against C. burnetii, were discovered in an area around Gooding, Idaho. Subsequent investigation in this area revealed the presence of 16 additional cases. All of these cases occurred in individuals working with sheep, and serological evidence of Q fever among sheep has been assembled. Studies are being continued.



Experimental epidemiological investigations of Q fever in cattle substantiate observations made on field animals in California. Aerosols containing 100 infectious guinea-pig doses per ml. are capable of infecting pregnant cows. This indicates that Q fever resembles brucellosis in cattle in this important aspect. The milking process has no effect upon the susceptibility of milking cows to Q fever, and in this respect the disease is dissimilar to streptococcal mastitis.

Attempts to infect cows by feeding them infected yolk-sac suspensions have produced negative results. Calfhood vaccination is being studied.

A comparison of the relative potency of certain drugs against infections of embryonated chicken eggs with C. burnetii has been made. The compounds may be arranged as follows in the order of descending effectiveness: terramycin, aureomycin, chloromycetin, streptomycin, and penicillin. In terms of molar quantities,  $1.6 \times 10^{-5}$  mM. terramycin,  $4.4 \times 10^{-4}$  mM. aureomycin, and  $7.3 \times 10^{-3}$  mM. chloromycetin were equally effective in preventing the growth of the organism. Penicillin had no significant effect upon the course of the infection.

Terramycin was capable of preventing fever or of reducing the length of the febrile period in guinea pigs infected with approximately 100,000 febrile doses of C. burnetii. The maximum dosage of terramycin necessary to prevent febrile response in the animals was 2 mg. maintained for a period of 3 days.

Pyruvic decarboxylase has been demonstrated in C. burnetii and R. prowazeki.

Two cases of typhus fever have occurred in Montana in an area where the disease is not endemic. One was in a Mexican who was probably infected when he crossed the border, and the other was in a Service man just returned from Korea.

A strain of spotted fever rickettsia was isolated from Dermacentor parumapertus collected from jack rabbits in Nevada. This is the first instance in which spotted fever infection has been found occurring in nature in this species of tick.

Indian tick-typhus from Kashmir, India, has shown the closest resemblance to boutonneuse fever immunologically and also, to a considerable extent, serologically. A yolk-sac vaccine prepared from this strain of typhus showed good serological activity, but failed to protect guinea pigs against challenge with a homologous strain. Indian tick-typhus has been passed through eggs to larvae of Rhipicephalus sanguineus, but trans-ovarial transmission of this rickettsial agent through D. andersoni has not been accomplished.

Ornithodoros turicata from Mexico is capable of transmitting Mexican spotted fever and also strains of spotted fever that are indigenous to the

United States. The same species of tick from the United States fails to transmit spotted fever to susceptible animals.

### Tularemia

Evaluations of specific vaccines against tularemia have been continued. Considerable controversy has been associated with the use of certain vaccines and certain animals for testing such vaccines. It has been argued that vaccines suitable for human use should be capable of protecting white mice, but consistently it has been demonstrated that none of the vaccines available will do so. By the simple process of challenging immunized animals intracutaneously, it has been possible to demonstrate that vaccines prepared from suspensions of Bacterium tularense extracted with diethyl ether are capable of protecting white mice against at least 200 and as many as 29,000 LD 50's. The data thus far assembled also show that immunized female mice are capable of resisting 10 to 40 times the number of infectious doses as are immunized male mice. Normal male and female mice are equally susceptible to infection with B. tularense.

The effect of cortisone on tularemic infections in white rats has been observed. It would appear that when rats infected subcutaneously with B. tularense are treated with cortisone the mortality rate is roughly three times as great as among rats that are infected but not treated with cortisone.

Antigen-antibody diffusion tests carried out with soluble antigens from tularemia organisms and high-titer antitularense serum show the presence of at least three components in the antigen under consideration.

Studies of an epizootic of tularemia in muskrats in Utah showed that at least 11 human cases of tularemia were associated with this outbreak among wild animals.

Sampling of natural waters in the Bitter Root Valley was initiated in an attempt to isolate B. tularense. Two streams are at present contaminated, and one of these appears to yield the organism continuously. An effort is being made to determine what factors are involved in initiating contamination of natural waters and in the continuing contamination of streams.

Surveys of sheepshearers in Montana and Idaho to determine the incidence of such infections as tularemia, brucellosis, Q fever, and spotted fever among persons engaged in this occupation are being continued.

Various cultures of smooth, rough, and indeterminate variants of B. tularense have been obtained from Camp Detrick. Extraction of suspensions of smooth and rough organisms with ether showed that smooth organisms contain large amounts of soluble antigens, whereas rough organisms contain only minimum amounts of extractable material.

## Plague

Soluble antigens occur in media and tissues in which Pasteurella pestis is present. These soluble antigens are relatively heat labile, and when tissues from animals having plague are boiled, the antigenic titer is greatly reduced or eliminated. Addition of ether to suspensions of such tissues serves the 2-fold purpose of killing viable organisms that are present and maintaining the antigen without decreasing the amounts of antigen as far as its reactivity in in vitro tests is concerned. The use of such suspensions of tissue in the diagnosis of plague in experimentally infected wild rabbits and rodents has shown that a modified Ascoli test may be of considerable value in the diagnosis of plague in field animals. By employing absorbed serums, it is possible to differentiate between infections due to P. tularensis, P. pestis, and P. pseudotuberculosis.

A medium containing low molecular weight compounds for the growth of P. pestis and the isolation of antigenic fractions from this organism has been developed. This eliminates the complication of solid media introduced by the addition of agar and will allow studies on antigenic fractions obtained by antigen-antibody diffusion tests, electrophoresis, and ultracentrifugation. The antigen of choice in immunizing mice against plague appears to be identical with that which has been isolated by Dr. K. F. Meyer's group and labeled as "Fraction 1." Employing antigen-antibody diffusion tests with this fraction, which is supposedly homogenous, it appears that at least three compounds are present in the fraction.

Studies on the chemotherapy of plague were continued. An intradermal test in rabbits was devised and employed. In this test the superiority of streptomycin against living P. pestis was confirmed. Aureomycin was capable of neutralizing plague toxin to a greater extent than streptomycin, terramycin, and chloramphenicol, and the combination of streptomycin and aureomycin was found to be most valuable in the protection of mice against infections with plague bacilli.

## Arthropods

Mosquitoes were collected from the Bismarck, North Dakota, and Hamilton, Montana, areas in association with studies of the virus encephalitides. Fourteen thousand specimens, constituting 21 species, were collected in the Bismarck area, and 16,000 specimens, constituting 24 species, were collected from the Hamilton area. In North Dakota a new westward distributional record was obtained for Psorophora ferox, an important pest species hitherto known to range from the eastern United States to South America. In Montana an apparently new species of Aedes was discovered, and a new distributional record was obtained for Mansonia perturbans, a pest species.

Three hundred ninety-one lots of chiggers, comprising 50<sup>+</sup> species, from the Western Hemisphere were identified, and many new distributional records were



obtained. Of this number, at least 30 new species of chiggers were identified, which suggests that our knowledge of this important parasite is extremely limited and emphasizes the need for a greater amount of work in this field.

A new and important westward distributional record was obtained for the man-infesting chigger, Eutrombicula splendens, from the Grand Coulee River region in Washington. This species was previously known only as far west as eastern Minnesota and eastern Texas.

Approximately 20,000 specimens of ticks, constituting 470 lots, were received for identification and study during the year. The previously unknown male of Dermacentor dissimilis, a species which has been found to be rather common on horses and cattle in southern Mexico and parts of Guatemala, was discovered and will be described. Ticks that are either Ornithodoros hermsi, an important vector of relapsing fever in certain western States or a very closely related species as yet undescribed, were discovered in Utah. Ixodes muris, a tick occurring on rodents in the eastern States, was found on muskrats in Utah for the first time. Ixodes baergi, previously known from birds in one area of Arkansas, was among materials sent to the laboratory from Colorado.

### Fungi

Surveys of native and domestic animals for Haplosporangium and Histoplasma infections were continued. Haplosporangium infections were reported for the first time in North Dakota, South Dakota, and Nebraska. An hemolysin has been isolated from H. capsulatum and some of the physical and chemical properties studied.

Actinomyces bovis has been studied with a view of growing this organism in liquid media employing quantitative methods. With the exception of possibly two factors, the identity of which is being investigated, the liquid medium is chemically defined. A toxin from Candida albicans appears to have an effect upon the intestinal tract and on the nervous system. Studies of this toxin are being pursued, since if this toxin is active in man, it may be of extreme importance because of the presence of this organism in various systems of normal human beings.

### Viruses

The survey of the occurrence of encephalitis in Montana and North Dakota was continued. No virus was isolated from mosquitoes, ticks, animals, or human beings in either of these States during the summer of 1950. These essentially negative results raise a serious question as to the importance of the mosquito-animal cycle in the maintenance of western equine encephalomyelitis and St. Louis encephalitis in nature.

Studies of viremia due to western equine encephalomyelitis virus in the blood stream of horses as related to intradermal, subcutaneous, or intravascular route revealed the surprising fact that it is extremely difficult to produce



viremia even though 200,000 LD50's of a recently isolated mosquito strain of the virus was employed. This would indicate that either mosquito strains differ in their growth capabilities as compared with laboratory strains or that mosquitoes are capable of injecting tremendous amounts of virus into animal hosts.

The virus isolated from Aedes trivittatus in North Dakota during 1948 and 1949 has been compared with the California virus isolated by Drs. Hammon and Reeves in that State. The two viruses are closely related, but do differ to some extent. To date, no clinical cases of infection caused by the trivittatus virus have been observed, although antibodies against this virus have been found in human and equine serums.

Investigations were continued on the adsorption of viruses to nonhost materials with the hope that the findings would prove applicable to purification procedures involving adsorption and elution or to chromatographic analysis of intact virus. It was found that substances present in the material to be analyzed for the specific virus influenced adsorption and that the salt content in the medium governed adsorption. The  $p^H$  played a minor role, although adsorption was at times less complete in alkaline media.

#### Schistosomiasis

The first laboratory confirmation of cases of schistosome dermatitis was made. Snails obtained from a pond at Ennis, Montana, were found to contain Schistosome cercariae, and an exposed individual developed typical lesions. In addition, two lakes in Flathead County, Montana, were found to contain infected snails.

#### LABORATORY OF CLINICAL INVESTIGATION

This laboratory - looking forward to the new Clinical Center facility - is now laying the groundwork for this Institute's clinical research program to be conducted in the Center. It will concentrate on infectious and parasitic diseases important to public health. Based on a coordinated laboratory - clinical approach, this program will seek to improve existing, or to develop new, methods and techniques for the diagnosis and prevention of infectious diseases, and to develop new or more effective methods of treatment, control or cure. Specific approaches will be employed on selected diseases along such lines as the early recovery, isolation and classification of etiological agents; the development of detectable antibodies of various categories; and therapeutic trials of certain drugs and antisera.

#### EXTRAMURAL PROGRAMS BRANCH

This operation, newly organized as a National Microbiological Institute function, has the responsibility for administration of research grants and research fellowships in the broad field of microbiology. This includes responsibility for such things as continuous review of disciplines and fields covered

by the NMI extramural program, coordination of such program with the several intramural research programs, maintenance of outside contacts with professional bodies and so forth.

The present extramural program covers the major fields of microbiological research; for example - infectious diseases, protozoal, rickettsial, spirochetel, and viral diseases, and investigations dealing with fungi and helminths. The program includes also, the sponsoring of fellowships to provide training for scientific personnel interested in microbiological disciplines. In addition, research grant support is implementing a nation-wide, sectional research program for biological warfare defense, the objectives of which are to expand and to strengthen the existing civilian facilities for the specific diagnosis of outbreaks of diseases not ordinarily included in routine laboratory techniques, and to encourage original research by independent investigators on diseases which may be of importance to biological warfare defense.

The support of this program has been made possible by the transfer of extramural funds from the Division of Research Grants. About one-half of such funds represented a "going" program to the extent of previously existing research grants committed to continuing support during the current fiscal year. On recommendation of the National Advisory Health Council in June, 1951, the Surgeon General awarded 66 additional research grants in the field of microbiology, totaling \$542,942. Approximately 48 institutions in 20 states and the District of Columbia were represented by the recipients of these research grants. Of the amount granted, an aggregate of \$26,400 was allotted to sectional laboratories in ten states for the purpose of initiating the research program for biological warfare defense.

The extramural programs Branch is conducting a survey of the appropriate study section members of the research grants program of the Public Health Service in order to determine the various fields of interest in microbiological research and to stimulate the development of neglected areas of study. A conference at which this subject will be discussed in detail has been scheduled by the Tropical Medicine Study Section for September 28-29, 1951; moreover, in connection with the promotion of advances in fields of major importance a symposium on toxoplasmosis is scheduled for the latter part of July or early August. A survey of the shortage fields within the Public Health Service fellowships program is also currently being made.

NATIONAL INSTITUTE OF MENTAL HEALTH

Robert H. Felix, Director

C O N T E N T S	Page
Introduction.....	149
Basic Research Laboratory.....	150
Addiction Research Center.....	151
Phoenix Mental Health Center.....	155
Research Grants & Fellowships Branch.....	156
Training and Standards Branch.....	161
Community Services Branch.....	163
Professional Services Branch.....	171
Biometrics Branch.....	172





NATIONAL INSTITUTE OF MENTAL HEALTH  
Robert H. Felix, Director

Introduction

Research in mental illness and mental health has been relatively limited in scope and dollar investment when compared with research in other health problems. Recognizing mental disorders as a public health problem, Congress passed the National Mental Health Act in 1946 to provide Federal support to research, training personnel in the mental health professions, and developing community mental health services. The first appropriation under the Act was passed in 1947 for the fiscal year 1948. The Division of Mental Hygiene administered the new program until the spring of 1949 when the Division was abolished and the National Institute of Mental Health established in the National Institutes of Health, the research arm of the Public Health Service.

The four parts of the National Institute of Mental Health concerned with the direction of the operating programs are the Office of the Scientific Director (intra-mural research), the Research Grants and Fellowships Branch (non-Federal research), the Training and Standards Branch (training grants and stipends) and the Community Services Branch (consultant services to States). The Biometrics Branch is concerned with the compilation and analysis of statistical data; the Professional Services Branch is concerned with developmental work on Institute programs; Publications and Reports Branch is responsible for the dissemination of scientific and technical information and mental health education activities of the Institute.

In the five years since the National Mental Health Act was passed, measurable progress has been made in the program. Intra-mural research is being conducted on a limited scale pending completion of the Clinical Center which will provide laboratory and bed space for clinical research. A cooperative agreement was signed during the year with the Worcester Foundation for Experimental Biology, whereby the Foundation and the National Institute of Mental Health are investigating the chemistry of adrenal steroids and the physiology of the adrenal cortex in psychotic and control subjects. During the year, substantial progress was made. Development of new methods of analyzing adrenalsteroids in the urine have permitted definite advances in knowledge of adrenal physiology.

Ninety-six research projects covering a wide range of study in the medical, biological, psychological and social sciences are being supported in universities, hospitals and other research institutions throughout the country. Prior to the passage of the Act, only 26 States and Territories had State-wide mental health programs. Today, the 48 States, the District of Columbia and the Territories all have "State-wide" mental health programs underway. While it is possible under

the Act to provide two Federal dollars for each State and local dollar expended for these programs, the ratio has now swung the other way and two State and local dollars are being expended for each Federal dollar, due to increasing State and local appropriations. The long-existing deficit in personnel trained in the mental health professions has been increased with the accelerated needs of the armed forces. One hundred and eighty-six training grants have been provided to universities, professional schools and other institutions for developing and expanding training programs in these professions and for providing stipends to graduate students. In addition 42 medical schools are receiving funds to develop psychiatric training for the undergraduate medical students. A Model Reporting Area has been established with a nucleus of eleven States participating, which will work on the use of uniform terminology in statistical reporting, and the development of more accurate, comprehensive and meaningful statistical data on the hospitalized mentally ill. The Federal funds expended during the fiscal year on this program totalled \$9,338,000 in round numbers.

During the fiscal year 1952, the regular program activities will continue, and certain additional work is being undertaken with the existing staff. One of the most important new activities will be the recruitment of scientific personnel for staffing the mental health research program at the Clinical Center which is scheduled for completion in the fall of 1952. With the appointment in May 1951, of the scientist who will direct the clinical and laboratory research of this Institute and the National Institute of Neurological Diseases and Blindness, the planning of the intra-mural research program is now being accelerated.

#### BASIC RESEARCH LABORATORY

##### Role of potassium ion in cortical reactions.

The role of potassium ion in cortical reactions has been studied with particular attention to role in spreading cortical depression and its general relation to polarization potentials of the cortex. We have found some striking similarities between time course and voltage swings of potassium chloride (KCl) and spreading depression. This work is being reported at the Fall Meetings of the American Physiological Society at Salt Lake City, Utah, September 5-8.

##### Compounds of striate cortex reactions.

Observations have been made on the components of striate cortex response to stimulation of optic nerve in answer to some criticism of previous work on this problem. The argument has been settled and some new information obtained by observing the effects of spreading cortical depression and KCl on the cortical components of the striate cortex reaction. This work is being reported at the Fall Meetings of the American Physiological Society at Salt Lake City, September 5-8.

### Role of acetylcholine in cortical reactions

The role of acetylcholine, topically applied to the cortex, has been investigated. It has been shown that some of the effects of acetylcholine previously described over a period of years is due to unrecognized spreading depression. These observations were reported at the spring meeting of the American Physiological Society, Cleveland, Ohio.

The laboratory has been considerably expanded and further additions made to our technical equipment in the past year involving procurement from outside sources and construction of specialized units in the laboratory.

### Chronic epilepsy studies in monkeys

A project on study of epilepsy has been initiated, and it is expected that this project will be continued indefinitely. Chronic epilepsy is produced in monkeys by placing ammonium hydroxide on the cortex or injecting same into cortex. The epileptic colony is well established and under intensive study. The number of preparations is limited only by animal room facilities. It is expected that this project will yield valuable information on the convulsive syndromes.

Considerable time has been spent on planning for the new Clinical Center laboratories.

### Studies on systems controlling overall activity of the brain

Tooling procedures for comprehensive investigation of subcortical ramifications of the reticular system and other systems concerned with overall physiological control of brain activity is practically completed.

ADDICTION RESEARCH CENTER  
Lexington, Kentucky  
Harris, Isbell, Chief

Operation of the Drug and Barbiturate Addiction Subproject during the current fiscal year was made difficult by increases in the prices of all types of supplies and equipment. We were able to maintain our operation at the usual level by depleting our stocks, and, fortunately, funds which became available during the last quarter permitted us to replenish them.

Administrative arrangements with the hospital have continued to be extremely satisfactory and we would, in fact, be unable to operate at all without the many valuable services furnished us by the Medical Officer in Charge and his medical and administrative staffs.

As judged by drops in the eosinophil count and the uric acid-creatinine ratio, the pituitary-adrenal system appears to be activated maximally during

abstinence from morphine. Neither the adrenocorticotrophic hormone (ACTH) of the anterior pituitary nor cortisone had any significant effect in ameliorating the morphine withdrawal illness. The response of the pituitary-adrenal system to injections of epinephrine and the adrenal response to injections of ACTH were not impaired after either single doses of morphine or during maintained morphine addiction, indicating that the pituitary-adrenal system is normal under both circumstances.

The status of the pituitary-adrenal system during barbiturate addiction and withdrawal is not as clear as during morphine addiction and the problem is still under study. The barbiturate abstinence syndrome is associated with a consistent rise in the blood non-protein nitrogen, and experiments designed to elucidate the mechanism of this phenomenon are under way.

Morphine decreases the anxiety generated by repeated painful electrical shocks delivered to the hand of human volunteers and prevents overestimation of the intensity of the shock. This effect explains in part the pain-relieving properties of the drug.

Bilateral prefrontal lobotomy does not decrease the intensity of abstinence from morphine, provided addiction is maintained for 1 to 4 weeks after the operation; prefrontal lobotomy does reduce the emotional reaction to withdrawal.

Morpholinylethylmorphine, which was studied at the request of the Expert Committee on Narcotics, United Nations, was shown to have a very low addiction liability.

A project designed to find a synthetic substitute for codeine was initiated during the year. The first compound studied, 2-methoxy-N-methyl-morphinan, has too great addictive properties to be considered as safe as codeine. Other compounds are to be studied in the immediate future.

Interest in drug addiction was more intense during the year in both medical and lay circles. A large number of speeches were delivered by members of the Research Branch and the films "Chronic Barbiturate Intoxication" and "Clinical Characteristics of Drug Addiction" were shown before a very large number of professional groups.



B.

## RESEARCH PROJECTS

No.	Title of Test	Number of Personnel		Date Initiated	Status 30 June 1951
		Professional	Sub-professional		
1	Physical Dependence in Schizophrenic Patients	3	8	July 1950	Continuing
2	Effects of Pre-frontal Lobotomy on Autonomic Reactions	1	1	July 1950	Completed
3	Conditioning of Vascular Reactions to Tetraethylammonium	1	1	July 1950	Discontinued
4	Effects of Barbiturates On Isolated Frontal Lobe of Humans	1	1	July 1950	Completed
5	Blood Sodium and Potassium Levels in Barbiturate Addiction in Animals and in Man	3	9	July 1950	In abeyance
6	Barbiturate Addiction in Dogs	2	2	July 1950	Continuing
7	Effects of Morphine on Decerebrate Dogs	2	2	July 1950	Completed
8	Effects of Morphine on Pupillary Reaction of Cats	2	2	July 1950	Completed
9	Effects of Morphine on Facilitation and Inhibition of Two-neuron Arc Reflexes in Acute Spinal Cats	2	2	July 1950	In abeyance
10	Effects of Large Doses of Morphine on Hindlimb Reflexes of Chronic Spinal Dogs	2	2	July 1950	Completed
11	Conditioning in Lobotomized Patients	4	2	July 1950	In abeyance
12	Relationship of Relief of "Anticipatory Anxiety" by Morphine to Analgesic Effect	4	2	July 1950	Completed
13	Addiction Liabilities of Acetyl Methadols	2	6	Aug. 1950	Completed
14	Effects of Morphine on Eosinophil Counts	2	6	Aug. 1950	Completed
15	Effects of Abstinence From Morphine on Eosinophil Counts	2	6	Aug. 1950	Completed
16	Effects of Pre-frontal Lobotomy on Abstinence from Morphine	3	9	Aug. 1950	Completed
17	Effects of Cortisone and ACTH on Morphine Abstinence Syndrome	2	6	Sept. 1950	Completed
18	Effects of Cortisone and ACTH on Barbiturate Abstinence Syndrome	2	6	Sept. 1950	Continuing
19	Effects of Large Doses of M-allylncetmorphine on Man	1	3	Sept. 1950	Continuing

B. RESEARCH PROJECTS (continued)

No.	Title of Test	Number of Personnel		Date Initiated	Status 30 June 1951
		Professional	Sub-professional		
20	Effects of N-allylnormorphine on Chronic Spinal Dogs	1	2	Sept. 1950	Continuing
21	"Acute" Addiction of Chronic Spinal Dogs	2	2	Sept. 1950	Completed
22	Effects of Morphine on Kohn Block Test	1	1	Oct. 1950	Completed
23	Effects of Abstinence From Morphine on Personality Traits as Measured by Projective Technics	1	1	Oct. 1950	Continuing
24	Effects of Morphine and Pitressin on Excretion of Water and Electrolytes by Kidney	3	7	Oct. 1950	In abeyance
25	Effects of Morphine Addiction on Urinary Excretion of Water and Electrolytes	3	7	Oct. 1950	Completed
26	Addiction Liability of Morpho-11nylethylmorphine	2	6	Jan. 1951	Completed
27	Human Pharmacology of Acetyl-methadols	2	6	Feb. 1951	Completed
28	Human Pharmacology of Isomers and Derivatives of 3-hydroxy-N-methyl-morphinan	2	6	March '51	Continuing
29	Mechanism of Morphine Miosis in Man	2	3	March '51	Completed
30	Comparison of Behavioral and Electroencephalographic Changes Induced by Drugs in Dogs and Humans	3	2	March '51	Continuing
31	Rapid Induction of Signs of Abstinence in Stabilized Morphine Addicts by administration of N-allylnormorphine	4	6	May 1951	Continuing
32	Reproducibility of Barbiturate Abstinence Syndrome in Man	2	6	Apr. 1951	Continuing
33	Addiction Liabilities of Isomers and Derivatives of 3-hydroxy-N-methylmorphinan	2	6	Apr. 1951	Continuing
34	Effects of Abstinence From Barbiturates on Blood and Urinary Nitrogen Components	4	8	Apr. 1951	Continuing

PHOENIX MENTAL HEALTH CENTER  
Phoenix, Arizona

The Phoenix Mental Health Center is a field station established to explore and evaluate methods of providing community mental health services and to study the ways in which various factors in individual, family and community life are associated with mental health problems. During fiscal year 1951 the staff curtailed clinical activities to a substantial degree in order to have more time to devote to the assessment of the program which had been carried out and to the development of techniques for this task. A major exploratory study on the school and family relationships and adjustment of children entering a public school was undertaken; it demonstrated the feasibility of securing for a sample of parents of school children data relevant to the process of adjustment and the ways in which family problems were handled. Subsequent studies will compare similar data secured for families seen at the Center with data on other families of school children.

A systematic interview survey was conducted among key personnel in city health, welfare and educational agencies to ascertain their reactions to the program at the Center. This survey revealed widespread disappointment that a greater volume of clinical service could not be provided, but enthusiastic praise for the consultation service provided various community agencies. Many workers in these agencies felt that the Center had been the most effective influence making for higher standards of performance by social agency personnel and had brought together and demonstrated the feasibility of cooperative work to agencies and organizations which had previously not worked together.

A major problem in the development of the Center's program has been the fact that community expectations and the past experience of Center staff members have been largely service-oriented. Since one aspect of the project involves the study of elements relating to the acceptance of a mental health program in a community, community pressures for service cannot be disregarded. On the other hand, the purpose of the Center is to provide communicable additions to knowledge. For this reason, a substantial proportion of staff time has been directed toward on-the-job training aimed at the development of the necessary tools for the research contemplated and the narrowing down of projects to the point where definite answers can be obtained for the questions asked.

Initial drafts of papers have been prepared describing and analyzing the operation of an interdisciplinary team in a community setting, presenting the findings of questionnaire survey of parents who participated in discussion groups operated by the Center and describing specific projects undertaken by individual staff members. The mapping of the distribution of health and welfare problems and other social phenomena has been proceeding steadily, as has the collection of basic demographic data for all families with whom the Center has established contact in any way. When data become available from the 1950 census, it should be possible to delineate with considerable accuracy the extent to which various

population segments - characterized by age, race, educational level, economic level, etc. - are being reached by the various facets of the Center's program.

#### RESEARCH GRANTS AND FELLOWSHIPS BRANCH

John C. Eberhardt, Chief

The Research Grants and Fellowships Branch is responsible for administering the extramural grant and fellowship program of the National Institute of Mental Health in the research field. As in the past, support has been given to a wide range of activities in the medical, biological, psychological, and social sciences. The detailed statistics regarding this program will be provided by the Division of Research Grants.

Over fifty research projects received continued support during the past year. Although most of these are long-term projects in which final results have not yet been attained, significant findings have already come from a number of them.

In the field of neuroanatomy, Dr. Conel's work at Harvard continues to furnish basic information regarding the postnatal development of the cerebral cortex. The study of the microscopic structure of the brain of the infant at the age of six months has been completed and the results published. Between the three and six-month stages of development, advances were noted particularly in the primary motor areas, especially in the region controlling the hand. This is correlated with the increase in voluntary motor control of the hand in the infant. Work is now going forward on the cortex of the infant at the age of 13-15 months.

Progress has been made in the field of neuropathology. Dr. Abner Wolf's studies at Columbia University on the etiology of cerebral palsy have clarified the pathogenesis of congenital toxoplasmosis, a protozoan infection which is one of a number of causes resulting in the clinical syndrome of cerebral palsy. Following up his previous work, in which it was found that this infection is acquired by the fetus from the mother, Dr. Wolf and his associates have shown that the organism reaches the placenta by way of the blood stream, producing lesions which, although not severe enough to interrupt pregnancy, permit the organism to enter the fetal blood stream.

The problems of the etiology and prevention of multiple sclerosis and neurotropic virus diseases are being attacked through study of an experimentally produced allergic encephalomyelitis in animals in the hope that the information obtained with regard to this experimental disease may be applied to the human diseases. Two projects in this field have been supported by grants from the National Institute of Mental Health. Dr. Elvin Kabat at Columbia University has recently found that a variety of complement-fixing antibodies is produced in the production of the experimental disease, but has found no evidence relating these antibodies to the disease. Progress in characterizing the nature of the substance which produced the disease is being made, it being found that the active



material may be lyophilized and extracted with benzene. Dr. Armando Ferraro at the New York State Psychiatric Institute has confirmed the earlier work of Bell, Hubel and co-workers that an allergic encephalomyelitis may be produced with a calcium acetate protein extracted from normal brain. Progress in the attempt to prevent the development of this experimental allergic encephalomyelitis has been made by using intramuscular injections of total brain proteins as a protective measure and by using injections of brain proteins from which the calcium acetate substance has been eliminated. Dr. Kabat has also shown that significant increases occur in the gamma globulin content of the cerebrospinal fluid in patients with multiple sclerosis, but not in the albumin content. The utilization of this fact for diagnostic purposes is now being developed.

Support has been given to a number of continuing studies on emotionally disturbed children. One of these, carried out by Drs. Redl and Lippitt of the University of Michigan and utilizing group treatment methods has resulted in a number of significant findings regarding the phenomenon of behavioral contagion in groups of children. It has been found that certain children in the group are recognized by the others as having a high prestige-status, and that these children are much more able to initiate group activities than those with a low prestige-status, many of whom recognize their low-status position and either are unable or make little attempt to be more accepted and influential. Those who are accorded a secure group status tend to be freer in their activity, are less preoccupied with problems of social relationships, and are not only more able to influence other children but are also more open to suggestions from others, although able to resist such suggestions if they wish. They are also regarded as more independent of adults and tend to be accorded the same high prestige-status in various situations, regardless of their ability to contribute in each situation. Certain of these children with high prestige-status, however, act as though they had a low status and, conversely, certain low-status children either do not recognize their position of low acceptance or refuse to admit it, thereby encouraging further rejection by the others. Dr. Lippitt proposes to utilize these data in an attempt to develop techniques for changing the group acceptance and social adjustment of disturbed children. A number of valuable methodological advances in the study of groups has also resulted from this study.

Another project dealing with the institutional treatment of emotionally disturbed children has been carried on by Dr. Bruno Bettelheim, Director of the University of Chicago's Orthogenic School. Dr. Bettelheim finds that "love is not enough" to successfully treat such children, according to the title of a new book recently published by him, and that many children manifesting severe behavior and personality problems can best be treated in a special environment, providing twenty-four-hour treatment. He stresses the importance of on-the-spot therapy in which day-to-day situations are dealt with at the moment they arise, as well as the importance of obtaining staff members with the requisite qualities for handling such children. Dr. Bettelheim plans to study the latter problem in greater detail in the next few years.

Over half a dozen projects have been supported in the field of child personality and development, four of which are long-term studies of the normal child. One of these, conducted by Drs. Roger Barker and Herbert F. Wright of the University of Kansas, is concerned with the naturally occurring behavior of children in a small American town and the relation of their behavior to the actual physical and social conditions under which they live. New methods of describing and analyzing the behavior and "psychological habitat" of children have been developed, representing an important contribution to the field of psychological ecology. Several publications have already resulted from this study, one of which, "One Boy's Day," presents a complete record of the behavior of a boy during the course of one day.

A second study, that of Drs. Leitch and Escalona of the Menninger Foundation, is concerned with the range and variability of normal infant behavior during the first seven months of life, rather than with establishing norms of behavior as has been done in previous studies, notably those of Dr. Gesell. Detailed data have been obtained and are now being analyzed in the following fields: the range and variability of infantile functioning in the sensory and gastro-intestinal systems and in the skin; the occurrence of behavior syndromes; the varieties and determinants of maternal attitudes; sleep patterns of normal healthy infants; maternal behavior in the feeding of infants; the relationship between psychological test findings and "adjustment states" in infancy. In addition, data on a number of special problems have been collected.

Of the several projects being supported in the field of psychosomatic medicine, one of the most important is that of Dr. I. Arthur Mirsky, formerly with the May Institute for Medical Research and now at the University of Pittsburgh. Dr. Mirsky has found that patients with duodenal ulcer have a significantly higher rate of pepsinogen secretion than healthy subjects, whereas patients with pernicious anemia excrete practically no pepsinogen. A highly significant increase has been found in the pepsinogen activity of the blood from patients with peptic ulcer and a decrease in that from patients with pernicious anemia or after gastrectomy. Subjects with high pepsinogen excretion rates appear to be very dependent personalities; those with pernicious anemia reveal the presence of a paranoid nucleus. These and other data suggest that there are individuals born with the capacity for hyper- and hyposecretion by the stomach and that the apparently specific psychodynamic constellation which may develop is the result of a sequence of events initiated by the physiological anlage.

Dr. Roy Grinker and his associates of Michael Reese Hospital have obtained data suggesting that the emotional state usually characterized by weeping may also find expression in other parts of the body, such as the skin, and that certain pathological edemas of the skin may develop as a result of a change in the emotional state of the individual.

In the field of therapy, Drs. Bowman and Ruesch of the University of California, in their study of social situations used in psychotherapy, have

found that non-verbal communication plays a far greater role in psychotherapy than has been previously recognized.

Drs. Alfred H. Stanton and Morris S. Schwartz of Chestnut Lodge Sanitarium have continued to produce new and important findings in the course of their psychiatric and sociological study of a mental hospital ward. A study of incontinence among psychotic patients shows that in some patients incontinence is a significant form of social participation; that it may represent a peculiarly appropriate response in the social context of a mental hospital ward; and that the extent to which it plays a part in the patient's illness is determined in part by the social structure of the ward, which often serves to perpetuate this type of behavior. This finding has both practical and theoretical significance, both for the care of the mentally ill and for an understanding of the contribution of the current social situation to autistic behavior. Another finding of this study was that, although the staff of a mental hospital ward may consciously work on the assumption that fulfillment of the patients' needs is one of its most important goals, this goal may in actuality be far from realized because of certain accepted traditions of interpreting patients' needs and behavior which are largely illusory in nature.

In the field of the relation of mental health and illness to social and cultural factors, Dr. Joseph Eaton of Wayne University has been carrying out a study of the mental health of the Hutterites, a small religious sect reported to have a very low incidence of mental illness. Dr. Eaton's study is still in progress, but in the course of evaluating the cultural and environmental factors which might explain the good condition of mental health among these people, Dr. Eaton has had to re-examine our concepts of mental health. In a recent paper, he has described five different criteria by which mental health may be evaluated, each with its own validity, depending on the context in which it is used. Dr. Eaton concludes that mental health "cannot be reduced to a single dimension," but is a value judgment, "with all the potentialities for variation and change implicit in such a relativistic entity," and that the "use of multiple criteria promises an advancement of knowledge beyond that achievable by a nonrelativistic approach."

In the field of forensic psychiatry, a survey of laws pertaining to the administration of mental institutions has been carried out by Dr. Leon Ehrlich of the National Association for Mental Health in all 48 States. The analysis and comparison of how these laws operate in actual practice will be carried out in the coming year and should have important practical application for State and local administrators of mental institutions.

In addition to continuing support for on-going projects, some 46 projects received support from the National Institute of Mental Health for the first time during the past year. As in the past, support has been given in a wide variety of fields, ranging from basic studies on the structure and function of the nervous system to a study of the value of a teaching program in human behavior and emotional development for children of different age levels and backgrounds.



Two new conferences to be held in the near future were subsidized by grants from the National Institute of Mental Health. One, on the effects of early experience on mental health, will be conducted by the Roscoe B. Jackson Memorial Laboratory in Bar Harbor, Maine. As a result of the efforts and interest of the Mental Health Study Section in clarifying the inter-disciplinary problems and techniques in the field of mental health research, a grant has been awarded to the National Training Laboratory for Group Development to conduct a series of work conferences on this subject in 1951 and 1952, in conjunction with the annual meetings of the major professional associations active in the mental health field.

In line with present interest in the effect of the present national emergency and its stresses and strains on our national life, a grant has been awarded to study the problems of family and personal adjustment which arise in a rapidly expanding community, resulting from the establishment of a new atomic energy plant by the Atomic Energy Commission. The relationship of social structure to psychiatric disorders will also be studied at Yale University. Two projects have received support to study the value and effect of specialized training and therapy for mentally handicapped children. As a follow-up of previous studies on psychosurgery supported in part by the National Institute of Mental Health, a grant has been awarded to investigate the post-operative social adjustments of patients who have undergone psychosurgery. In addition to two continuing projects in the field of perception-personality relationships, a 5-year program in this important field has been initiated at Clark University through support from the National Institute of Mental Health. Both the Worcester Foundation for Experimental Biology and the Neurological Institute of New York have received grants to study the effect of ACTH and Cortisone on psychiatric and neurological disorders, primarily schizophrenia and multiple sclerosis. Two new studies in the field of epilepsy have received support, as well as a study of the possible value of a preventive mental health program for aged people. Analysis of the central nervous system by means of the electron microscope, as well as a number of other studies on the anatomy, physiology, and chemistry of the brain have received support. Support has been given to over half a dozen projects in the field of experimental psychology, in which animals will be used to evaluate the role of such various factors as early infantile deprivation, anoxia, electro-convulsive shocks, and cerebral lesions on later behavioral and neurological disorders. These and other projects supported by the National Institute of Mental Health are indicative of the wide interest in the problems of mental health and illness and the variety of methods being used to attack them.

An increased number of fellowships over the previous year were awarded in 1951. These fellowships were awarded at both the predoctorate and postdoctorate level and are for the purpose of furthering the training of promising young research investigators. As with the research grant program, support has been given to investigators in a wide variety of fields, biological, medical, social, and psychological, with the greatest emphasis on postdoctoral training



in the fields of neurophysiology and psychology. A considerable increase of interest in the fellowship program of the National Institute of Mental Health was evidenced, and the number of applications increased to such an extent that a number of promising investigators could not be awarded fellowships because of insufficient funds.

TRAINING AND STANDARDS BRANCH  
Seymour D. Vestermark, Chief

During the fiscal year 1951 a considerable part of the operational activities of the National Institute of Mental Health continued to center about training in the field of mental health. There was, and still remains, an urgent necessity to continue these training activities because of the deficit of mental health specialists to meet the increasing mental health needs of the country and to provide skilled personnel to meet the needs of the armed forces. Demands for personnel to meet these needs will be even more acute during this period of partial national emergency. In addition to providing assistance for individual professional training, aid has been made available to promote the further development and expansion of graduate teaching programs in the areas of psychiatry, clinical psychology, psychiatric social work, psychiatric nursing, public health mental hygiene, and neurology. Until additional funds become available to the N.I.N.D.B., the National Institute of Mental Health will continue to support to a limited extent training in the field of neurology.

Recognizing the importance of mental health in the field of public health, the National Institute of Mental Health has made funds available to three additional schools of public health during the fiscal year 1951 to aid in the development of divisions of mental health in these graduate schools. This is significant in that it will provide expanding opportunities for the training of the general public health officer as well as for those desiring specialized mental health training in the field of public health.

The table below indicates the amount of funds made available under the National Mental Health Act during the fiscal year 1951 for graduate training in the various specialty fields:

1951 Grants - Graduate Training

	<u>Traineeships</u>		<u>Teaching Grants</u>		<u>Total</u>	
	<u>No.</u>	<u>Amount</u>	<u>No.</u>	<u>Amount</u>	<u>No. of Grants</u>	<u>Amount</u>
Psychiatry	169	\$443,232	56	\$749,017	65	\$1,192,249
Neurology	8	22,200	7	55,681	7	77,881
Public Health						
Mental Hygiene	2	7,500	5	43,998	5	51,498
Clinical						
Psychology	103	198,131	43	392,515	52	590,646
Psych. Social						
Work	126	221,634	35	374,031	36	595,665
Psychiatric						
Nursing	177	294,530	21	300,796	21	595,326
Totals	585	1,187,227	167	1,916,038	186	3,103,265

In addition, funds totaling \$499,015 were made available during the fiscal year 1951 to 42 medical schools to assist them in the improvement and further development of undergraduate psychiatric education. Unfortunately, it was not possible to provide assistance to the remaining 37 medical schools because of limited funds. Information received from these 42 medical schools reveals that these grants have been provocative in improving undergraduate psychiatric education. The funds supporting this aspect of training were part of a three-year block grant made to 42 medical schools in 1950 which expires in 1952. It is anticipated that additional funds will be made available to continue this undergraduate program from 1953 on, on a yearly basis.

During the year the National Institute of Mental Health made six special grants in the field of mental health totaling \$41,150 to hold institutes in public health mental hygiene for public health officers and nurses and psychologists. One of these special grants was made to the Department of Psychiatry, Yale University, in order to evaluate the effectiveness of this type of institute as a teaching device. A grant was made to the Department of Institutions and Agencies in the State of New Jersey to conduct an institute on civilian morale and mental hygiene. This latter will have eventual significance in the mental health aspects of planning for civil defense.

The National Institute of Mental Health made a grant to the American Psychiatric Association and the Association of American Medical Colleges, who jointly sponsored a conference on undergraduate psychiatric education held at Cornell University, Ithaca, New York, June 21-27, 1951. This conference was unique in that it brought together psychiatric educators and deans from selected medical schools, together with leaders from other disciplines concerned with human relationships, such as biology, social sciences, law, the ministry,

psychology, public health officers and representatives from general practice who met for the purpose of exploring all phases of undergraduate psychiatric training and the broader implications of the preparation of the general practitioner to meet the emotional needs of the community. A second conference on graduate training under the same sponsorship is being planned for June, 1952.

COMMUNITY SERVICES BRANCH  
James V. Lowry, Chief

The program of the Community Services Branch of the National Institute of Mental Health includes assistance to the States in developing community mental health programs, demonstrations, mental hospital survey and consultation services, State mental health facilities surveys, special studies, cooperative activities with national public and private organizations concerned with mental health activities, and psychiatric and psychological services to the Juvenile Court of the District of Columbia.

The National Advisory Mental Health Council has a Community Services Committee with 15 members from all parts of the United States. The members are authorities in the field of mental and public health and act as an advisory group through the National Advisory Mental Health Council on the Community Services program of the National Institute of Mental Health. This committee met October 19 and 20, 1950.

During fiscal year 1951, the Community Services Branch had 33 professional staff members on duty - 8 at headquarters in Bethesda, 17 in the Regional Offices, 6 at the Prince George's County Mental Health Clinic, and 2 at the Juvenile Court of the District of Columbia.

ASSISTANCE TO STATES PROGRAM

The States are assisted in their development of community mental health programs by providing professional consultation from the Regional Offices and by making grants-in-aid to the States. Each State has an agency that is responsible for administering the federal grant-in-aid funds for mental health services. In 34 States the agency is the State Health Department; in 6 States, the Department of Mental Hygiene; in 5 States, the Department of Welfare; and in 8 States, other Departments. During 1951, legislation in 2 States changed the agency administering the grant-in-aid funds from the State Health Department to the Department of Mental Health in one instance, and to the State Department of Welfare in another. Twenty-nine States utilize councils of professional and lay members as advisory groups in their mental health programs.

The amount allotted to any particular State is determined by the population of the State and the per capita income. The minimum grant for any State in fiscal year 1951 was \$20,000. The grant-in-aid funds were utilized to establish mental health services other than those related to patients in hospitals and/or institutions.

During fiscal year 1951, all of the States and Territories had a mental health program. This is in contrast to the situation that existed in 1946, when 24 of the States and Territories had a State mental health program. The program in some of the States is quite well developed, and in others is rudimentary. In fiscal year 1951, \$3,200,000 was available from new appropriations for grants-in-aid to the States. There remained in the State treasuries from fiscal year 1950 about \$350,000, making a total of \$3,550,000 available for expenditure during fiscal year 1951. During the past several years there have been increased amounts of State and local funds, both public and private, expended for mental health services. During fiscal year 1951, \$3,502,685 of federal grant-in-aid funds were budgeted. The State plans for the utilization of this money included provision for concomitant expenditure of \$5,258,516 of State public funds, \$2,038,434 of local public funds, and \$1,182,725 of private funds, making a total of \$11,982,360. From this, it can be seen that the State and local funds were equal to approximately twice the amount of Federal funds in the programs.

The activities of the State mental health programs can be grouped into several general categories--central administration, program administration, professional services, clinic services, preventive and educational services, and training of personnel. The number of States that included these various items in the program and the percentage of federal grant funds budgeted by activity is as follows:

	Number of States	Percent of Federal Grants
Clinic Services	46	60 percent
Mental Health		
Education	33	13   "
Program Administra-		
tion	36	10   "
Training	39	9   "
Professional Services	19	5   "
Central Administra-		
tion	31	3   "

Since many of the clinics supported provide consultation services, professional services, educational activities, and other community functions in addition to providing treatment services to individuals, some of the funds that are budgeted under clinic services contribute to other areas of the program.

Grants-to-States funds were utilized in the support of 330 mental health clinics. Of these, 172 were in operation prior to 1947 when grant-in-aid funds first became available and the grants permitted the expansion of services in these clinics. One hundred and fifty-eight of the clinics were established as a direct result of the availability of federal grant assistance. During 1951,



22 new clinics were started. The average number of patients seen in a clinic was 300 and each patient was seen an average of six times. (These figures are based on reports from 153 of the 330 clinics.) In 1951 the staffs of the clinics continued to increase the amount of their time that was used for community mental health activities. These included consultation and educational services to community agencies such as schools, courts, health and social agencies.

Increased attention in State programs is being given to preventive and educational services. Almost \$450,000 of federal grant funds were budgeted for this purpose. One of the principal methods for broadening the application of mental health services has been the in-service training of community agency personnel by means of short-term institutes. One hundred and twenty-four of these institutes were held in 26 States. These institutes were designed to improve the ability of personnel to assist individuals with emotional problems in order to prevent their developing a disabling illness. The people in attendance at institutes included health officers, teachers, nurses, physicians, ministers, social workers, Juvenile Court personnel, parents and rehabilitation personnel. In order to reach large numbers of people, many of the States utilized motion pictures, printed material, radio, and other audio-visual materials. Mothers of first-born children were sent monthly informational materials over a period of a year on the physical and emotional development of the child. There has been a significant expression of appreciation for this material by the mothers. Many states have established circulating libraries to provide physicians and other professional workers with books and journals. A psychiatric bulletin began publication this year and is being distributed to physicians in general practice in several of the States.

During the year some State agencies have been able to employ additional qualified professional personnel to administer the mental health program. However, some States have found it impossible to do so because of the shortage of personnel and in some instances because of the low salaries offered. The staff in the State agencies proceeded with the development of the mental health programs in spite of the difficulties associated with new programs, personnel, and fund shortages. In addition to the program in their own agency, they stimulated and assisted other State and local agencies in developing mental health services.

Professional services - psychiatric, psychological, psychiatric social work and nursing - were provided to schools, courts, health, welfare and other community organizations. These personnel provided services to individuals with emotional problems, consultation to organization personnel, and in-service training.

One of the chief difficulties in establishing and carrying out mental health activities is the shortage of trained professional personnel. Many of the States, therefore, found it necessary to give stipends to individuals who agreed to return to employment in the mental health program after completion of their training. About \$300,000 was budgeted for stipends for 131 trainees.

This included 29 in training in psychiatry, 33 in clinical psychology, 62 in psychiatric social work, and 7 in mental health nursing. After the training of these individuals is completed, it will permit the State programs to bring services to additional people.

#### DEMONSTRATIONS

The Prince George's County Mental Health Clinic is operated as a joint activity with the Maryland State Department of Health and the local County Health Department. It is a demonstration project designed to carry on a preventive program in conjunction with the local health department. In this capacity it has as its objective the study of how a community can protect the mental health of its citizens and each activity of the Clinic is evaluated with this objective in mind. Prince George's County has a population of nearly 200,000 which is partially urban and partially rural. The Clinic is located in quarters furnished by the University of Maryland because room was not available within the County Health Department. The amount of mental illness in a community can be decreased by decreasing the number of mentally ill and by decreasing the incidence of mental illness. In order to achieve this goal, it is recognized that treatment of existing disorders, particularly when other individuals are affected, is the first step in controlling illness and preventing illness. In addition to this, it is necessary for the general principles of mental health to be known and utilized by all who are in a position of responsibility, particularly those who are dealing with children, or have responsibility for public health. Beyond this, of course, it is necessary that all citizens understand the importance of decreasing emotional strain in every day living, in order to preserve mental health. The Clinic's staff works both with individuals who are treated in the Clinic and in community activities, and staff time is divided about equally between these two general types of activities. Information about clinic activities was made available through publications and visitors from 19 States and 9 foreign countries came to the Clinic during the year to obtain first-hand information.

In regions I, IV-V, VII and VIII, the mental health consultants participated in meetings with representatives of the State agencies administering the mental health programs. Meetings were held in the Regional Offices and were attended by one or more persons from each State. The discussions proved valuable in stimulating an exchange of information between States on program activities problems, and future program. The State personnel have requested that additional meetings be held.

The principal responsibility for assisting the States lies with those personnel assigned to the Regional Offices. Their function has been particularly valuable in terms of furnishing professional consultation with regard to the organization and administration of mental health programs. As programs develop beyond the initial stages, these personnel are called upon more and more frequently for consultation in their special field of training. Without

personnel in the Regional Offices, it would have been impossible for the State programs to move forward as rapidly as has occurred.

Members of the Clinic staff have met with groups of professional and lay personnel in discussions of mental health topics. By this means, the Clinic staff had direct contact with over 2,000 people in the community. These people, by and large, are the ones who come in contact with large numbers of the population in their daily work. Several demonstration projects on specific aspects of mental health have been carried forward during the year. In cooperation with the County Department of Education, a study was made of the inclusion of mental health information and symptoms of pupil maladjustment in the Health Manual for the use of teachers. Another activity with Junior High School teachers was a study of the recognition of problems of adjustment of school children. Continued assistance was provided to the schools in their work with special reading problems and remedial reading classes. Much of the time of the Mental Health Nurse was spent in the County Health Department prenatal and well-baby clinics in group work with parents and with individuals. One project of significance was the work with the nurses of the Telephone Company with regard to industrial mental hygiene. The work of the staff includes much work with parents. For instance, in one high school the study of vocational problems of students led to the organization of a Mothers' Discussion Group concentrating chiefly on how to help their high school youngsters mature toward eventual vocational and personal adjustment.

During the year, 377 individuals were seen at the Clinic - 164 adults and 213 children under the age of 18. Twenty-five percent of the people who came to the Clinic did so after hearing about the work of the Clinic through friends or family, articles, or meetings; twenty-five percent were referred to the clinic by local physicians; the remainder were referred from schools and other community agencies. Three hundred and two patients ended their contact with the Clinic during the year. One hundred and fifty-three had 1 to 3 hours of Clinic time, 96 had 4 to 9 hours, and 53 had more than 10 hours of Clinic time. The severity of the problem rather than the age of the patient appeared to be the principal determinant of the number of hours of treatment necessary. The Clinic was able to give definite help to 80% of the patients. In 19% of the 377 cases there was marked improvement resulting in a better job, school or home adjustment, or in the alleviation of a situation giving every indication of being a forerunner of a serious mental disorder. In working with the individual patients, it was apparent that health, education, and social agencies, etc. can play a major part in preventing mental health problems, and it is therefore essential the Clinic staff work closely with these groups.

The members of the demonstration project staff are called upon for activities outside of the County. In the past year, members have been taking part in mental health institutes in Massachusetts, Maryland, and Pennsylvania.



## HOSPITAL CONSULTATION SERVICES

The headquarters staff of the Community Services Branch includes a psychiatrist and psychiatric nurse whose function is to survey and make recommendations for the improvement of hospital and institutional care of the mentally ill. The requests for such surveys and consultation almost always come from the governor of a State and are based on the need for evaluation of the care and treatment of patients in hospitals, and recommendations with regard to the improvement of such care. The completed surveys and recommendations are almost invariably used in determining legislation action designed to improve mental hospital care. During the past fiscal year, surveys were done of 23 psychiatric hospitals and related institutions in Colorado, Illinois and South Dakota.

## SPECIAL STUDIES

In October 1950 the Community Services Committee appointed a Subcommittee to prepare a report on mental health implications in civil defense programs. A member of the headquarters staff served as Executive Secretary of this Subcommittee. The Subcommittee was composed of three representatives of the Community Services Committee living in the same general geographical section of the country. The Subcommittee and the staff members from the Community Services Branch met in California, in order to formulate the report. This report has been completed and will be presented to the Community Services Committee. Following this, it can be utilized by Regional Mental Health Consultants in their work with the States. The report itself includes an introduction formulating basic principles and the reasons for mental health in a civil defense program. The next section is on psychological implications in planning emergency care, and includes consideration of combatting fear and panic, care of emotional casualties, care of mental hospital patients. The third section is on psychological concepts pertinent to understanding civilian needs and leadership training. This includes community problems as they affect emotional stability, motivation and apathy, preparation of the individual for stability in disaster, placement of children in time of disaster, public information, training, and administration. The fourth section contains recommendations. The report is well annotated with over 40 references, and in most instances, abstracts of pertinent reference material is included.

Narcotic drug addiction, particularly in individuals under the age of 21, received considerable interest during the past year. A conference attended by representatives from various parts of the Federal Security Agency and mental health personnel from the Regional Offices was held June 28 and 29, 1951. Prior to the meeting, the Regional Office personnel obtained all available information on narcotic addiction in the regions. This information indicated that narcotic drug addiction in individuals under 21 was principally a problem in the deteriorated areas of large cities and particularly among the Negro population. Arrangements have been made to furnish consultative services to States and cities by utilizing personnel of the National Institute of Mental Health, the Hospital



Division, and the Hospital Facilities Division of the Public Health Service. As a sequel to the meeting, the Federal Security Agency established an intra-agency committee on drug addiction.

The special grant to the University of Illinois to develop screening techniques that will identify individuals in need of further diagnostic study was continued during the year. The first phase in the development of such screening devices has been completed and the work continues to further develop the methods and to test them. Provision has been made for the continuance of this project during fiscal year 1952.

The basic field work for the first survey of mental health facilities of the 48 States and the District of Columbia was completed in 1950. In the final work-up of the accumulated data, certain additional information was obtained during fiscal year 1951 because of recent developments of significant proportion. In Massachusetts, this amounted practically to a complete resurvey, due in part to a marked increase in psychiatric services which became available in general hospitals throughout the State. It may be desirable to repeat this country-wide survey by States in 1955, to compare with the present base line data.

During the past year, in cooperation with State and local agencies, a study of alcoholism as a public health problem has been undertaken to obtain information for program development. Twenty-five of the thirty-six currently operating State programs have been visited. These programs vary greatly, not only in motivations, objectives, legislative and financial support and administrative auspices, but also in relative effectiveness. Practically all of them, except those concerned with preliminary surveys, include treatment and rehabilitation among their objectives. When case finding and early treatment methods can be developed, and appropriate educational efforts included in the program, many of the State programs will possess the cardinal elements of the recognized public health approach. A roster and summary of State programs and a comprehensive file for current data concerning overall activities in the field of alcoholism, on federal, State and local levels, has been set up.

These activities have laid the groundwork for a more substantial cooperative participation with the various State and local agencies working in the field of alcoholism, and assistance has been given to several State organizations. Working relations have been effected with the National States Conference on Alcoholism, the National Committee on Alcoholism, the Committee on Alcoholism of the National Research Council, and the Industrial Hygiene Division and Hospital Division of the Public Health Service. A special grant was made to the Yale Center of Alcohol Studies for a survey. The National Institute of Mental Health has been designated as the focal point within the Service with regard to alcoholism.

Activities related to mental health problems of aging were begun during the year. Visits were made to community facilities in Maine, Ohio and New York,

as the initial step in obtaining information for program planning. One of the staff members served as the mental health member of the Secretariat of the health section of the National Conference on Aging held in Washington in August of 1950. The Branch was represented in conferences on aging held during the year in Ann Arbor, Michigan and St. Louis, Missouri.

#### COOPERATIVE ACTIVITIES

The staff of the Community Services Branch, both at headquarters and in the Regional Offices, has worked cooperatively with other constituencies of the Federal Security Agency and the national voluntary agencies on those aspects of their programs having mental health significance. In cooperation with the Office of Education of the Federal Security Agency, a Report of Conference on Mental Health in Schools and Teacher Education Institutions was published. This report discusses the in-service education of teachers and other school personnel; pre-service preparation of teachers and other school personnel concerned with mental health problems; administrative planning to assure a healthy mental atmosphere in the schools; developing and improving the school program to meet the mental health needs of school children of all ages; home, school, community cooperation, parent education working relations with other community resources; the use of specialized mental health services by schools and teacher-education institutions; evaluation of mental health needs, resources, and programs in the school. This report brings together material from a conference held in 1949 which was attended by psychiatrists, psychologists, cultural anthropologists, school social workers, school teachers, principals, superintendents, teacher training personnel, and other special consultants to the National Institute of Mental Health and the Office of Education.

In addition to work with the Office of Education, headquarters personnel worked with the Industrial Hygiene Division of the Public Health Service, the Children's Bureau and the Office of Vocational Rehabilitation of the Federal Security Administration. The principal voluntary organizations where liaison was maintained were the National Association for Mental Health, the Family Service Association, the National Social Welfare Association, Red Cross, Community Chests and Councils, and the National Urban League.

#### JUVENILE COURT

The Juvenile Court of the District of Columbia operates clinic services as an integral part of the Court, and a psychiatrist and psychologist are assigned to this operation. This clinic furnishes diagnostic and therapeutic services to patients referred to it from the Social Service Department of the Court, and recommendations are made regarding the disposition and handling of the case. The personnel work with the children and their parents since, in order to be helpful to many children, intensive work with the family is required. During the fiscal year 167 cases were referred to the Clinic for consultation and treatment. An additional function of the Clinic staff is their participation

in the training of graduate students in clinical psychology from Catholic University and George Washington University. Pediatric internes from Children's Hospital attend clinical conferences at the Juvenile Court Clinic during the period when they are in the psychiatric service at Children's Hospital. The clinic staff lectured at St. Elizabeth's Hospital, the District of Columbia Health Department, and Catholic University during the year. There is a close working relationship with the Mental Hygiene Clinic of the District Health Department. The psychiatrist served as consultant to the Social Service Guild of St. Luke's Episcopal Church and the staff cooperated in the development of the "Conference of Metropolitan Mental Hygiene Clinics."

#### CONCLUSION:-

This report has, in general, indicated accomplishments that are significant. However, they are small in comparison with the job that remains to be done. The reduction in appropriations from \$3,550,000 in 1950 to \$3,200,000 for 1951 resulted in a curtailment of program activities and in some States personnel had to be released. The cut in appropriations to \$3,100,000 for 1952 will further curtail programs. In addition to this the States have become apprehensive about further fund reductions and are reluctant to plan and initiate further development toward an adequate program.

#### PROFESSIONAL SERVICES BRANCH

Joseph M. Bobbitt, Chief

During 1951 the Professional Services Branch continued to carry out its general functions in the field of developmental work on Institute programs, supervision and development of special projects and consultation within the Institute and to other parts of the Service. Branch personnel was depleted by virtue of the fact that both the psychiatrist and psychiatric social worker were in training status for most of the year and the mental health nurse was assigned to another Branch for the last half of the year. \*

Developments worthy of note included the exploration, by Branch personnel, of the field of industrial mental hygiene and human relations in industry. A panel of consultants to the Institute was assembled to discuss the status of knowledge in industrial mental hygiene and to suggest possible program developments on which the National Institute of Mental Health and the Division of Industrial Hygiene might collaborate. Subsequent to this meeting, major program developments in implementing the suggestions of panel members have been made by the Division of Industrial Hygiene.

Attention was devoted to the problem of drug addiction among juveniles and young adults and toward the close of the fiscal year the Professional Services Branch was given the responsibility for planning the Institute program in this field. Special research grants to study the distribution and sociological aspects of the problem in New York and Chicago were arranged by Branch personnel.



Consultation and coordination of work proceeding within a number of the States on the evaluation of mental health activities, including training institutes, was undertaken at the request of outside groups concerned with such evaluation. Preliminary plans were drawn up for the design of training institutes which would permit more adequate evaluation than has heretofore been possible.

Other activities carried out by Branch personnel included the administration of the Phoenix Mental Health Center, staff work for the Mid-Century White House Conference on Children and Youth, and representation of the Institute on the Mental Health Film Board, in conferences sponsored by the Josiah Macy, Jr. Foundation relating to human relations in Germany, in a series of conferences sponsored by the New York Academy of Medicine relating to matters of panic prevention and civil defense, and on various Agency committees.

BIOMETRICS BRANCH  
Morton Kramer, Chief

During the past fiscal year several new projects were started by the Biometrics Branch for the purpose of achieving greater standardization in the collection of statistics on the hospitalized mentally ill and to obtain more extensive data.

Under the sponsorship of the National Institute of Mental Health a group of mental hospital administrators and statisticians, representing 11 States that provide care for 55 percent of all patients in State mental hospitals, met in Washington on February 26 - 28 to discuss how more accurate statistics could be obtained on the hospitalized mentally ill. This meeting was the first of its kind to be held in this country. The 11 States represented were Arkansas, California, Illinois, Louisiana, Michigan, Nebraska, New Jersey, New York, Ohio, Pennsylvania and Virginia.

The purposes of the conference were: (1) to define the kinds of statistical data currently needed on the mental hospital population of the United States, (2) to explore the possibility of standardizing definitions of terms so that inter-State comparisons might be more valid, (3) to explore research possibilities of data collected on the State mental hospital population, and (4) to explore the possibility of setting up a Model Reporting Area for mental hospital statistics.

Both administrators and statisticians indicated their specific needs for statistical data and pointed out the inadequacy of the type of data now being collected. From the administrative point of view, major emphasis was on the need for personnel standards, such as setting up ratios of patients to specific types of personnel.

The development of the new nomenclature and statistical classification, under the auspices of the American Psychiatric Association was outlined, and



there was discussion of the possible effects of the introduction of this nomenclature on mental hospital statistics.

The National Institute of Mental Health proposed that the conference group constitute itself a Model Reporting Area for mental hospital statistics. In view of the obvious benefits to be derived from the establishment of such an Area, the conference group agreed to form the nucleus of a Model Reporting Area, and will aid in establishing and maintaining standard definitions, tabulations and classifications to achieve more uniform and meaningful statistics. Other States will be encouraged to develop and improve their statistical systems and eventually to become part of the Model Reporting Area.

The conference passed specific recommendations on trial use of certain definitions and adoption of the new nomenclature by mental hospitals. Standing committees on definitions, tabulations and statistical classification of psychiatric disorders were created to expedite the establishment and aid the functioning of the Model Reporting Area.

In an effort to gather data in an area in which there has been a conspicuous lack of information on a national basis, the National Institute of Mental Health sponsored a study in 1949 among the 11 States mentioned above to obtain data (1) on the composition by age, sex, diagnosis and length of stay for first and readmissions among resident hospitalized patients, discharges and deaths in State mental hospitals (no information of this type on a national basis has been available since 1939) and (2) on the disposition of patients during the 12 months following their first admission. Progress was made in clarifying concepts relating to the collection and presentation of uniform data in this area.

PUBLICATIONS AND REPORTS BRANCH  
Alberta Altman, Chief

In anticipation of the expansion of the intra-mural research program and the opening of the Clinical Center, Publications and Reports Branch stressed the research aspects of the Institute program. Increased efforts were made to acquaint interested professions, science writers and the general public with the research activities. Special publications, speeches and papers were prepared in cooperation with members of the professional staff on a wide range of subjects including drug addiction, alcoholism and aging, and an increased number of reprints of scientific and other professional papers were obtained for distribution.

An active program for the dissemination of information about the policies and procedures of the Institute continued, and materials dealing with the principles of human behavior and personality development received nation-wide distribution through radio and television programs, films and magazine articles. This report is not a listing of activities but attempts to point out the major projects completed during the year.



NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC  
DISEASES

Russell M. Wilder, Director

C O N T E N T S	Page
Introduction.....	177
Laboratory of Biochemistry and Nutrition.....	177
Section on Biochemistry and Physiology of Nutrition.....	177
Section on Fractionation and Isolation.....	180
Section on Enzymes and Metabolism.....	181
Endocrinology Section.....	184
Laboratory of Chemistry.....	184
Section on Carbohydrates.....	184
Section on Analgesics.....	185
Section on Steroids.....	187
Laboratory of Pathology and Pharmacology.....	189
Section on Comparative Pathology and Hematology.....	189
Section on Metabolic and Degenerative Diseases.....	192
Section on Pathologic Anatomy.....	198
Section on Pharmacology and Toxicology.....	202

	Page
Laboratory of Physical Biology.....	204
Section on Molecular Biophysics.....	204
Section on Low Energy Radiation Biology.....	206
Section on Nuclear Radiation Biology.....	206
Section on Physiological Physics.....	207



## Introduction

To promote more extensive research in the field of arthritis and rheumatism -- chronic illnesses that afflict an estimated seven to eight million persons in this country -- the National Institute of Arthritis and Metabolic Diseases was created November 22, 1950.

In few fields is the need for research more acute. According to insurance statistics, nearly 9 percent of all sickness and 15 percent of absence from work among wage earners are due to arthritis. It is the world's greatest crippler.

In preparation for its expanded responsibilities, the new Institute has appointed a chief for clinical research and a chief for extramural programs. Limited programs have been launched in each of these areas.

### LABORATORY OF BIOCHEMISTRY AND NUTRITION James M. Hundley, Chief

The Laboratory of Biochemistry and Nutrition is organized to conduct both fundamental and applied research in the broad fields of biochemistry and physiology. The Section on Fractionation and Isolation searches for and isolates chemical substances from natural sources such as vitamins, growth factors or other substances of biological importance. The Section on Enzymes studies the fundamental chemical processes by which an organism derives energy from its food and isolates and studies the enzymes and substrates involved. The Section on Endocrinology studies the influence of the endocrine glands on metabolic processes and various disease states as well as the interrelations between the central nervous system and the activity of the endocrine glands. The Section on the Biochemistry and Physiology of Nutrition studies the actions, uses and biochemistry of vitamins and amino acids, the role of nutrition in various disease processes, searches for new vitamins and other substances of possible medicinal importance. The overall program of the laboratory is planned to emphasize research bearing on arthritis and allied diseases, peptic ulcer, diseases of the liver and blood forming organs, particularly the biochemical and physiologic aspects of these subjects.

### SECTION ON BIOCHEMISTRY AND PHYSIOLOGY OF NUTRITION

A dietary deficiency of copper or pantothenic acid and, in some cases, a deficiency of lysine produces gray hair in black rats. In the

pantothenic acid deficient rats, the graying is associated with marked increases in the copper content of the skin. Large amounts of ascorbic acid in the diet will prevent the graying due to a deficiency of pantothenic acid; glucuronic acid is even more effective than ascorbic acid in that respect. The gray hair resulting from a copper deficiency appears earlier in male than in female rats. When male rats on a copper deficient ration are castrated, adrenalectomized or hypophysectomized, the gray hair becomes black again. Hypo- or hyperthyroidism reduces the grayness of the copper deficient rats while the addition of zinc, cobalt or ascorbic acid to the diet accentuates it.

Nicotinic acid deficiency in rats is accompanied by high fasting blood levels of lactic acid and a diabetes-like blood sugar curve following a large dose of glucose. Sodium bromide is not a nicotinic acid antimetabolite for rats as has been reported for man in the clinical literature. Studies are under way on the excretion of nicotinic acid-like compounds by dogs fed 3-acetyl pyridine.

When rats are fed a low protein diet containing 3 percent ferric citrate, they grow slowly and develop a marked granulocytopenia with varying degrees of leucopenia and anemia. Iron salts other than the citrate produce the same result. Folic acid prevents or corrects the blood dyscrasia but has no influence on growth. Casein and certain amino acids partially prevent or delay the effects of the high iron feeding. The blood disturbance is associated with the presence of iron in the gastrointestinal tract since the injection of large amounts of iron has no influence on the blood.

Rats fed large amounts of polished rice grow better when threonine and lysine are added to the ration. The amount required is approximately one-eighth that previously deemed necessary. The addition of other amino acids, singly or in combination, produced no greater growth than that secured with lysine or threonine.

The addition of valine, methionine or threonine to a stock ration depresses the growth of rats by 30 percent; the addition of any other amino acid retards growth by 10 percent.

The injection of DOCA, estradiol or saline solution improved the life expectancy and electrolyte and water balance of the heart in thiamine deficient rats.

The level of calcium in the diet had only a slight effect on the development of ascorbic acid deficiency in guinea pigs. When ascorbic acid was removed from the ration, the animals on the low level of calcium continued to grow a few days longer than did those on the high calcium intake. There was, however, no difference in the survival time of the

two groups. The anemia present in scorbutic guinea pigs was studied in some detail; these animals had no ascorbic acid in the adrenals and only very small amounts in the kidney and liver.

When large amounts of ascorbic acid are added to the diet of rats, it is able to partially replace either pantothenic acid, riboflavin or thiamin for growth. Ascorbic acid has no influence on the rats' requirement for pyridoxine. The ascorbic acid appears to be effective only when given by mouth.

In rats, the addition of 2 mg. aureomycin per 100 gm of a pantothenic acid deficient ration overcomes, to a certain extent, the deficiency; a level of 20 mg prevents the appearance of any deficiency signs which a level of 100 mg of aureomycin per 100 gm of ration produces as good growth as optimal levels of pantothenic acid. Small additions of aureomycin to a nicotinic acid deficient ration promotes growth in rats when sucrose is used but depresses growth when dextrin is used as the sole source of carbohydrate. Chloromycetin has the same influence as aureomycin. Antibiotics have less or no influence on the requirement of rats for thiamine or vitamin A. The addition of aureomycin to a diet results in the synthesis of citrovorum factor or the conversion of folic acid thereto. The daily injection of citrovorum factor into mice did not protect them from lethal doses of x-rays. The toxicity of small doses of aminopterin can be prevented by the addition of extra amounts of folic acid to the diet whereas the effect of larger doses of aminopterin cannot be overcome by folic acid. Aminopterin appears to inhibit the transformation of folic acid to the citrovorum factor. The citrovorum factor is destroyed by gastric juice.

The decreased gastric secretion of pylorus-ligated rats raised on a purified diet has been explained partially. Addition of meat meal or wheat bran to the purified diet produces an increased secretion while corn bran and a commercial non-nutritive filler had no influence. The decreased gastric secretion of pyridoxine or pantothenic acid deficient rats could be overcome by feeding large amounts of the appropriate vitamin 18 hours prior to the ligation of the pylorus.

When thyroidectomized rats were injected with growth hormone, there was an increase in body weight which was not associated with a comparable increase in muscle mass. The growth hormone stimulated the formation of skin and muscle collagen. Growth hormone is not able to stimulate muscle formation in the absence of the thyroid hormone. Force-feeding the thyroidectomized rats produces an increase in body weight and skeletal size but the increase in body weight is largely due to the deposition of fat. Thyroidectomized rats show a decreased nitrogen retention.

Male rats develop dietary liver degeneration earlier than females. A new dietary factor different from Vitamin E or cystine has been found to prevent liver degeneration.

In the presence of desoxypyridoxine, the growth of E. Coli bacteriophage is decreased. The inhibition of bacteriophage growth can be overcome by the addition of acetate and a number of other metabolites to the medium. Under these circumstances the growth of bacteriophage does not result in the uptake of any of the carbon atoms of the acetate molecule.

A number of new compounds related to tryptophane have been synthesized. They will be studied to see if they can act as metabolic antagonists and thus aid in the elucidation of the physiological role of tryptophane.

A fairly high percentage of older people show a stomatitis of the buccal mucosa. In some cases, it is associated with a leukoplakia which is occasionally a precursor of cancer. A study is being made to see whether both the stomatitis and the leukoplakia can be cured by nutritional means.

For many years, it has been known that many children show their greatest weight gains in late fall and early winter while no weight gain or actual loss of weight occurs in late spring or early summer. There are many reasons for associating the differential weight gains with variations in the nutritional situation. An attempt is being made to see if the intake of certain nutrients, or the blood levels of such things as vitamins A and C, carotene and hemoglobin are related to the differential growth of children.

#### SECTION ON FRACTIONATION AND ISOLATION

The section concerns itself chiefly with the isolation and characterization of new and unidentified substances present in natural materials important in nutrition and growth. It consists of three parts (1) the fractionation and isolation unit, (2) large scale laboratory, and (3) nutritional microbiological assay unit. The chief problems underway during the last year have been the isolation studies of the citrovorum factor and investigations on its biochemical functions, the isolation and characterization of the urinary glutamic acid derivative from rats on a folic acid deficient sulfa-containing diet and collaborative microbiological assays. The program for the coming year will be the further extension of these problems which are underway.

It has been possible to demonstrate that the citrovorum factor which we have obtained as highly purified concentrate is more active than the



synthetic tetrahydro-formyl-pteroyl glutamic acid which is believed by others to be the citrovorum factor. In addition the natural product differs from the synthetic in its behavior on treatment with acid. The project has as its ultimate goal the isolation in pure form of the citrovorum factor, its chemical and biochemical characterization. Intensive studies leading to the isolation of the conjugated citrovorum factor will be carried out. The excess urinary glutamic acid in folic acid deficient rats has been shown to arise from an unknown substance which on heating is converted to glutamic acid. The program for the coming year includes its final purification and identification.

"Influence of Succinylsulfathiazole and Folic Acid on Glutamic Acid Excretion" by H. A. Bakerman, M. Silverman and F. S. Daft, J. B. C. 188, 117-123, January, 1951.

"Comparison of Citrovorum Factor and a Synthetic Compound with Leuconostoc Citrovorum Growth Activity" by M. Silverman and J. C. Keresztesy, J. Am. Chem. Soc. 73, 1897, 1951.

#### SECTION ON ENZYMES AND METABOLISM

During the past year the work of this section has resulted in an understanding of the biological degradation and synthesis of a number of important intermediates in nucleic acid and coenzyme metabolism, including nicotinamide mononucleotide, adenosine-5-phosphate, and pyrophosphate. Reactions discovered for the degradation of pyrimidines by bacterial enzymes furnish valuable clues to the possible mechanism of biosynthesis of these substances. In the oxidation of carbohydrate, a new intermediate, ribulose-5-phosphate, has been identified and its enzymatic conversion to ribose-5-phosphate established, disclosing an important link between carbohydrate metabolism and nucleic acid synthesis.

Study was continued of the fundamental chemical processes by which an organism derives energy from food and the means by which this energy is used for growth and function, including the study of the enzymes and intermediary metabolites which result from enzyme action. The activities of the past year have included investigations of the degradation, structure and biosynthesis of components of nucleic acids and nucleotide coenzymes, and of the oxidative pathway of carbohydrate metabolism. These projects will be continued in the coming year.

A crystalline enzyme has been isolated from yeast which specifically catalyzes the hydrolysis of inorganic pyrophosphate, and this enzyme is being used in a study of the metabolism of pyrophosphate. It has been established that pyrophosphate can arise from the hydrolysis of ATP (adenosine triphosphate) by enzymes purified from several animal tissues. The enzyme which specifically hydrolyzes 5-nucleotides has

been obtained in highly purified form and used in the identification of naturally-occurring nucleotides.

The phosphorolysis of nicotinamide riboside has been demonstrated with an enzyme purified from liver and the reaction shown to be reversible. All the steps have now been demonstrated by which DPN (diphosphopyridine nucleotide) can be formed from its components, except for the formation of adenosine. The breakdown and synthesis of this substance is now under investigation. The cleavage of DPN has been studied with isotopic pyrophosphate and the mechanism of the reaction clarified. The structure of TPN (triphosphopyridine nucleotide) has been established.

A highly specific enzyme has been purified from rabbit muscle which phosphorylates adenosine and 2,6-diaminopurine. Enzymes have been discovered in bacteria which convert uracil to barbituric acid and thymine to 5-methylbarbituric acid. The further degradation of these products by bacterial enzymes is being investigated.

The oxidation of 6-phosphogluconate with a purified enzyme from yeast has been shown to result in the formation of the ketopentose, ribulose-5-phosphate, identified for the first time. An enzyme has been discovered which catalyzes the conversion of this ester to ribose-5-phosphate in a reversible manner. Preliminary results with isotopic carbon indicate that the first step is also reversible, leading to the fixation of  $\text{CO}_2$  in phosphogluconate. The same series of reactions has been shown to occur in a number of animal tissues. The further metabolism of the pentose phosphates is being investigated.

A new method for the purification of DPN and TPN by ion-exchange chromatography has been developed.

#### ENDOCRINOLOGY SECTION

This section is concerned with problems which deal with the physiological aspects of endocrinology. One of the chief research projects is the relation of the central nervous system (CNS) and particularly the hypothalamus to the function of the endocrine glands and to metabolism. Although the importance of investigating this problem has been recognized for half a century, the field is just beginning to be explored. The recent rapid advances in endocrinology such as the isolation of many of the hormones, some in a "pure" state, and the development of satisfactory assay methods sensitive to micrograms of the hormone have furnished new techniques which can now be used in testing the functional capacity of the endocrine glands under various neurological conditions. Such questions as: what is the main spring in the mechanism of the pituitary-adrenal axis, and what is the

homeostatic mechanism concerned with the metabolism of protein, carbohydrate and fat, are being subjected to experimental investigation.

In carrying out this study animals are being prepared with various neurological lesions. In dogs:- low cervical cord transection, transection at the midbrain level, isolated lesions in the midbrain and hypothalamus and decortication. In cats:- decortication, and isolated lesions in the hypothalamus and thalamus. Metabolic studies related to protein, carbohydrate and electrolytes and water are being made in the dogs. Hormone studies are being made in both dogs and cats. In order to measure hormones in blood and urine the following methods of assay are being developed; in the blood:- thyreotrophic hormone, ACTH and adrenal steroids; in urine:- adrenal steroids. Additional assay methods to be set up are: in blood antidiuretic hormone, insulin and possibly adrenalin.

The mode of production of mucopolysaccharides is being investigated through a study of hexosamine metabolism. Acute non-specific stress causes a transient rise in serum hexosamine levels. We have demonstrated that this initial rise is abolished in the stressed hypophysectomized rat. This suggests that a pituitary hormone is the factor responsible for the serum hexosamine changes.

ACTH lowers elevated hexosamine levels so it is not the responsible hormone. The same is true of cortisone. Further, stressed-adrenalectomized rats also get rises in hexosamine. Thyrotrophic hormone can reproduce the phenomenon. Stressed-thyroidectomized rats get an exaggerated rise in hexosamine levels. Thus the mechanism of stress-induced hexosamine increases is apparently an effort of thyrotrophic hormone.

Further evidence indicates that in the absence of this hormone the body hexosamine stores are depleted (liver, skin, and muscles).

It is planned to devote particular attention to more exactly defining this new hormone system by developing a liver-hexosamine assay method and to study the interrelationship between this system and the other endocrine systems. Further studies on the body metabolisms of hexosamines are planned.

Incidental to this work, since the thyroid contains appreciable amounts of hexosamine it is hoped that another new assay may be developed for thyrotrophic hormone. Evidence already suggests that this assay may be practicable.



LABORATORY OF CHEMISTRY  
Lyndon F. Small, Chief

The primary function of the Laboratory of Chemistry is to carry out fundamental and applied researches in chemistry as related to problems of biology, medicine and public health. It also cooperates with other units of the National Institutes of Health in projects which can benefit from its organization and experience. The present researches relate to carbohydrates, analgesics and steroids. They are planned to be continued in the coming fiscal year. This laboratory is organized into three sections: Section on Carbohydrates, Section on Analgesics and Section on Steroids. In addition, three services (administrative, scientific and technical) are attached to this Laboratory: The chairmanship of the National Institutes of Arthritis and Metabolic Diseases Editorial Board, the Survey of Chemotherapeutic Agents, a liaison between the Chemical-Biological Coordination Center of the National Research Council and the National Institutes of Health, and the Analytical Service Laboratory, serving all the laboratories of the National Institutes of Health, which may require certain kinds of precise analytical work.

SECTION ON CARBOHYDRATES

The principal objective of the Section on Carbohydrates is to investigate the fundamental chemistry of the sugars and their derivatives with a view to developing new and improving old methods of synthesis in order that members of this series may become more accessible for medical research. In pursuance of this objective the program of the Section may be considered as consisting of the following three projects.

The synthesis of keto sugars through the action of Acetobacter suboxydans on sugar alcohols. -

The action of A. suboxydans on the heptitols  $\beta$ -sedoheptitol and D-gluco-D-ido-heptitol has afforded two new sugars, L-guloheptulose and D-idoheptulose; much of the chemistry of these new sugars has been elucidated. This project is continuing.

The synthesis of higher-carbon sugars. -

Studies of the higher-carbon sugars were continued with the synthesis of the new sugar D-alto-D-gluco-heptose from D-altrose. This project is continuing.

The synthesis of sugar alcohols and their derivatives. -

It has been found that various sugar and sugar acid derivatives may be reduced by the new reagent lithium aluminum hydride to sugar alcohols. This



reductive method is of value for the preparation of sugar alcohols and for the identification of sugar derivatives. In continuation of this project an investigation of various cyclic acetals for the synthesis of sugar alcohol derivatives has been begun.

## SECTION ON ANALGESICS

### Basic chemical investigations. -

Systematic: A novel preparative method for the partial demethylation of tertiary amines has been elaborated. The reaction is limited through certain, well defined structural factors.

Alkaloids: A study of the reduction of various strychnos alkaloids by lithium aluminum hydride. All were reduced to tertiary amines in the expected manner, except brucine which furnished a dehydro derivative. It was observed that codeinone undergoes an acid-catalyzed hydration, to form 8-hydroxydihydrocodeinone. An investigation to determine the relative configuration of atropine and cocaine has been undertaken.

Dihydrothebaine has been degraded in a novel manner to a dimethoxytrialkyltetrahydronaphthalene (?). This opens a possible way to a complementary proof of the point of attachment of the ethanine system at C-13 in the morphine alkaloids. These studies are being continued.

### The synthesis of potential chemotherapeutic agents. -

A series of amino- and guanidino-pentadecylphenyl glucosides and a number of *p*-aminobenzoyl and *p*-aminosalicyloyl derivatives of meso- and epinositol were synthesized for in vitro testing on Dubos-Davis medium (H37Rv) by the Tuberculosis Research Laboratory, U.S.P.H.S., Cornell University Medical College. This project is to be discontinued.

### Chemical and pharmacological studies with analgesics. -

Three new series of analgesics have been developed: 1,1-diphenyl-2-methyl-2-dimethylamino propanol-1, 1,1-diphenyl-2-methyl-3-dimethylamino propanol-1 (and O-acyl derivatives), 1,1-diphenyl-3-dimethylamino butanol-1 (and O-acyl derivatives) 1,1-diphenyl-3-dimethylamino butene-1. In these series only weak analgesic effects were observed.

The acetyl-methadols. The methadones may be reduced in two distinct ways. To the  $\alpha$ -methadols (LiAlH<sub>4</sub> or Pt) and to the  $\beta$ -methadols (with sodium). These methods were extended to the optical isomers dextro- and levo-methadone leading to the optically active members of the  $\alpha$  and  $\beta$  series. All methadols thus obtained were converted to their corresponding acetyl derivatives. These complete series allowed a stepwise correlation of

structural features and changes with physiological effects (e.g. toxicity, analgesic power). Two findings appear important: First, the change from the ketone to the alcohol diminished markedly analgesic activity and subsequent acetylation, restored analgesic potency to or above that of the precursor ketone. Second, striking and peculiar differences in the euphoric response to  $\alpha$ -acetylmethadol, levorotatory, and  $\alpha$ -acetylmethadol, dextrorotatory. This consisted in a higher effectiveness, prolonged action and more rapid onset of effect on oral, as compared with parenteral administration, with the  $\alpha$ -acetylmethadol, levorotatory, (derived from d-methadon). These studies are being extended to the isomethadone series.

Pharmacological studies included further: Morphine derivatives with nuclear substituents, degradation products of dihydro thebaines (both stereoisomers of 3,4-dimethoxy-6-dimethylamino-13-ethyl octahydrophenanthrene show a moderate analgesic effect), and 1,1-di( $\alpha$ -thienyl)-3-dialkylamino butanes and the corresponding 1-butenes submitted by the Wellcome Research Laboratories.

N-Allylmorphine. During the year the antagonism of N-allyl-normorphine to the analgesic effect of morphine was explored. The former abolishes, or prevents, the analgesic effect of the latter when administered approximately simultaneously in a ratio of at least 1 part of N-allyl to 10 parts of morphine. Since it has already been reported that N-allyl antagonizes the toxic and respiratory effects of morphine and other analgesics, a study was initiated of the possible use of this substance to antagonize the depressant effect of morphine and other analgesics on the infant when these drugs are used for obstetrical analgesia. It was demonstrated that N-allyl, given to the mother after a dose of morphine or other analgesics sufficient to depress respiration in the fetus, would restore the normal respiratory activity in the fetus, or, given before morphine or other analgesics, would prevent such respiratory depression. Work was initiated on the antagonism of N-allyl to the effects of morphine in an addicted individual for its possible relation to the mechanism of abstinence. It was demonstrated that N-allyl, in a ratio of 1 part to 10 parts of the morphine to which the individual was accustomed, would almost immediately precipitate a typical morphine abstinence picture.

These observations may have very important practical applications, both of which are being explored. A regime may be worked out whereby N-allylnormorphine can be used in human obstetrics to prevent the often disastrous depression of the fetus when drugs are used for analgesia in the mother. It may also be possible to use N-allyl as a quick test of the degree of addiction in a suspected individual.

Enzymatic studies. -

Methadone derivatives were found to be active inhibitors of pseudo and specific cholinesterase but not in the same order as their analgesic activity.

Over a limited range the solubility of CO<sub>2</sub> in phosphate buffers is proportional to pH and CO<sub>2</sub> pressure but practically independent of ionic concentration.

Red blood cell cholinesterase was obtained in soluble form by avoiding precipitation of "ghosts" with acid.

SECTION ON STEROIDS

Plant sources. -

Cardiac glycosides: Two samples of Strophanthus seeds (collected in Portuguese West Africa) were found to be unusually rich in one glycoside (ca 1%) which was elucidated as sarverogenin diginoside (apparently identical with Reichstein's compound No. 761). The second minor glycoside is apparently identical with Reichstein's No. 762.

For future rapid analysis of seeds a micro method for the qualitative determination of the glycosides (applicable to a small number of single seeds) has been developed. It consists of a modification of the Schindler-Reichstein paper chromatographic method. Resolution is effected by benzene-formamide or the toluene-propylene glycol system. The development of spots is effected with Tollens' reagent or trichloro acetic acid. The analysis of the remainder of Strophanthus seeds supplied by the Department of Agriculture will be conducted in this manner (primarily for determination of sarmentogenin content).

Steroidal alkaloids: Tomatidine (by hydrolysis of the glycoside tomatine) has been degraded to  $\Delta^{16}$ -allo-pregnen-3 $\beta$ -ol-20-one, thus opening a possibly industrial route to hormones (e.g. testosterone, progesterone, desoxycorticosterone and cortisone).

Solasodine, the aglycone of solasonine (extracted from berries and seeds of Solanum aviculare, S. auriculatum and S. xanthocarpum from Madagascar, New Zealand, and India) has been degraded to 3 $\beta$ -acetoxy- $\Delta^{5,16}$ -pregnadiene-20-one.

Experiments are under way towards the elucidation of the nitrogen-containing moiety in tomatidine as well as in solasodine.

Partial synthetic procedures. -

Synthetic experiments in the "neo-ergosteryl" series were continued and extended. These derivatives were subjected to hydrogenation and oxidation under a variety of conditions. The photo-oxidation of the "7-dehydro steroids" to the "bis-compounds" was applied to bis-norcholadienates and dehydro-diosgenin. Studies are under way to convert ergosterol derivatives (dienes) to suitable precursors for the synthesis of cortisone.

Total synthetic experiments. -

Experiments are being continued, aiming at the synthesis of as-octahydrophenanthrene derivative substituted in the appropriate positions to allow the construction of 11-oxygenated perhydrogenated cyclopentanophenanthrenes.

Analytical and metabolic studies. -

For use in experimental animals and humans, micro-procedures were set up for the determination of neutral 17-ketosteroids (modified Callow and Emmens method) and of neutral formaldehydogenic lipids (modified Corcoran and Page method). The application of the former to dogs has shown a surprising lack of ketosteroids in dog urine, even after stimulation by ACTH. With the object of developing a method for the detection and estimation of minute quantities of individual ketosteroids, corticosteroids and estrogens in biological mixtures a series of paper chromatographic studies has been made. A paper chromatographic procedure for the identification of cardiac glycosides and genius has been devised and work on the chromatography of minute quantities of steroids and sapogenins in progress.

Services. -

Survey of chemotherapeutic agents

The Survey Office of Chemotherapeutic Agents serves primarily as liaison between the Chemical-Biological Coordination Center of the National Research Council and various laboratories of the National Institutes of Health. The number of compounds processed was 591. These were obtained primarily from the Chemical-Biological Coordination Center, further from laboratories of the National Institutes of Health, academic and industrial laboratories and from miscellaneous private sources. Laboratories of the National Institutes of Health cooperated in testing compounds for toxicity, effect upon enzymes, analgesic action, tumor necrotising effect, fungicidal and molluskacidal qualities and for their value against experimental malaria, schistosomiasis and virus infection. The Tuberculosis Research Laboratory of the Public Health Service (located at Cornell University



Medical College) cooperated in the tuberculosis tests. The results of 456 tests were communicated to this office.

### Service Analytical Laboratory

A total of 5057 micro- and semimicro analytical determinations have been carried out (C, H, N, Halogens, active hydrogen, metalloids and metals, etc.). Service is extended to all laboratories of the National Institutes of Health requesting certain types of analytical work.

### LABORATORY OF PATHOLOGY AND PHARMACOLOGY

Ralph D. Lillie, Chief

The principal function of the Laboratory of Pathology and Pharmacology is to investigate the morphologic and immunochemical aspects of natural and experimentally produced degenerative, metabolic and infectious diseases, both independently and in cooperation with other constituent Institutes of National Institutes of Health, to determine the chemical nature of substances seen in sections of normal and diseased tissues and devise improved technics for their demonstration, and to study the effect of chemo-therapeutic agents, antibiotics and certain metabolites on normal and diseased animals. Administratively the laboratory is divided into four sections in accordance with the principal fields of research of the groups as follows:

Section on Comparative Pathology and Hematology

Section on Metabolic and Degenerative Diseases

Section on Pathologic Anatomy

Section on Pharmacology and Toxicology

Their work is not mutually exclusive but each provides consultation and assistance to other sections and units of National Institutes of Health as the development of various research problems may require. The projects occupying the several sections during the past year and planned for the future are described below by sections:

### SECTION ON COMPARATIVE PATHOLOGY AND HEMATOLOGY

One unit of this section conducts research work on bacterial, viral, and parasitic diseases of laboratory animals to enable investigators to distinguish between the lesions produced experimentally from that produced by the spontaneously occurring diseases in the NIE animal colonies, and to develop effective methods for the control or prevention of these natural occurring diseases. .

One unit of this section conducts research work on the pathogenesis of radiation injury.

A major activity of this section is to examine and evaluate the pathologic lesions of laboratory animals produced in the course of research work in other laboratories of the National Institutes of Health.

A study of the protective effects of glutathione in radiation injury. This study lends some support to the notion that a humoral factor exists which stimulates bone marrow regeneration after radiation injury.

Histopathologic studies of experimental arthritis in albino rats and mice with Corynebacterium spp and Streptococci spp. (with H. Friedlander).

Evaluation of the effect of the common cold virus on mice and rats was made and no significant lesions were observed.

Three different molluscacides given to calves were found to be non-toxic to the calves.

A report on the behavior of the Newcastle virus and the resulting pathological lesions caused in infected suckling and adult mice was completed in cooperation with Drs. Kilham and Peers.

Studies of liver necrosis, liver cirrhosis, experimental rheumatic fever attempts, and sulfone toxicity in animals (in collaboration with others).

Determination of the incidence of spontaneous diseases in laboratory animals of the NIH animal colonies by recording gross and microscopic lesions, bacteriological and parasitological findings.

Outbreaks of diseases in laboratory animals have been investigated and methods for their control have been suggested.

Small colonies of mice, rats, guinea pigs, and rabbits are maintained under strict sanitary conditions and various methods of control and treatment of the spontaneous diseases in these animals are evaluated, such as: coccidiosis in rabbits, lymphadenitis in guinea pigs, middle ear infection and bronchiectasis in rats, external and internal parasites of small laboratory animals, and Bartonellosis in rats.

Evaluation of medicaments for the control of salmonellosis in mice, rats, and guinea pigs.

Rats have been shown to survive otherwise lethal doses of radiation if joined in parabiosis to litter-mates soon after exposure to x-ray. The

increased survival appears due to accelerated bone marrow regeneration of the irradiated rat; possibly the non-irradiated animal supplies to the irradiated twin a substance necessary for regeneration of hemopoiesis from undifferentiated mesenchymal cells. Such a principle, if it can be conclusively shown to exist, may eventually be isolated and be of benefit in some of the aplastic anemias or agranulocytosis, as well as in the treatment of radiation injury.

Histopathologic studies of radiation injury have been continued with the general purpose of this investigation being to get a better insight into the mechanism of death after x-ray doses of a few hundred to a few thousand r.

Two distinct causes of death, pancytopenia at lower doses, and severe enteritis (denudation of small gut mucosa) with higher doses have been described in detail. The regenerative capacity of the small bowel mucosa has been studied in exteriorized, locally irradiated loops in 2 dogs and this investigation is being continued. Similar studies in other species are under way.

The study of parabiotic rats has defined more clearly the so-called "hemolytic component" and that "unexplained" anemia is probably due to internal hemorrhage, the extent of which has been underestimated in the past. These leads are being followed up with quantitative techniques.

The previously developed technique for accurate platelet counts, results of which have been confirmed by Carl V. Moore and others, have enabled us to follow the fate of transfused platelets. Platelet counts in guinea pigs have been doubled with a transfusion technique developed by Dillard. This work is being extended to dogs, a donor colony having been established by Dr. Thorp.

A study of the pathogenesis of the extremely virulent tubercle bacillus strain D-114 in animals was undertaken in cooperation with Dr. Emmart of the Laboratory of Pathology and Pharmacology.

Successful transmission studies of Western equine encephalomyelitis virus to horses at the Rocky Mountain Laboratory, Hamilton, Montana were initiated this spring. Tissue preparations of these animals are now being studied.

An agent obtained from aborted equine fetuses has been transmitted to guinea pigs for three separate passages. An evaluation of the pathological lesions caused and more complete transmission efforts will be made.

An extensive study of the pathology caused by Rous Sarcoma I virus in chickens is being made.

## SECTION ON METABOLIC AND DEGENERATIVE DISEASES

The program of this section has been directed toward the evaluation of the biochemical responses of man and animals to disease and other stresses. The role of the macromolecular components of blood, urine, and other tissues in these responses has been of primary interest to the group. The isolation and characterization of several proteins, lipo-proteins, polypeptides and polysaccharides of physiological importance has occupied one major group within the section. The biological fate and physico-chemical interactions of another group of proteins, polysaccharides, and formed blood elements has been the principal interest of another group. In order to carry out the isolation of macromolecular compounds especially those occurring in low concentrations e.g. as in urine it has been necessary to devise improved methods for handling large volumes of the source material under conditions which do not lead to denaturation or other destruction of these substances. Similarly with measurements of the metabolism and interactions of these substances it has been necessary to develop some special methods for measuring these. By combination of these methods it is hoped that it will be possible to measure the rates of formation, metabolic destruction and sources of these substances in relation to both normal and pathological states in man and animals.

The Section on Metabolic and Degenerative Diseases is composed of the following personnel:

Dr. Robert R. Williams	- Internist & Immunochemist, Acting Chief
Dr. Harry A. Saroff	- Protein Chemist
Dr. William C. Knox	- Immunochemist
Dr. Joseph H. Bragdon	- Pathologist and lipid Chemist
Dr. George H. L. Dillard	- Internist & Protein Chemist
Dr. Filadelfo Irreverre	- Biochemist
Dr. Arthur T. Ness	- Organic and Isotope Chemist
Dr. Elijah Adams	- Internist and Enzymologist

In addition to the above:

Dr. Gert Laqueur - Endocrine Pathologist - has been working in Pathological Anatomy. His activities will be reported with this section.

Dr. Kenneth M. Endicott - Pathologist - has left the group and been assigned to the Research Grants and Fellowships Division.

Dr. Robert E. Smith - has resigned to become Associate Professor of Physiology at the Medical School of the University of California at Los Angeles.



Dr. William C. Knox will be leaving the group October 1 to take a residency in pathology.

The senior investigators are assisted by two junior professionals, four technicians and a section secretary.

During the past year the activities of the section have centered around main groups.

Drs. Saroff and Dillard have largely concentrated their attention on the development of methods of isolation and separation of proteins, polysaccharides, polypeptides and other smaller molecular weight substances complexed with these substances. For this purpose they have completed the development of a large scale membrane diffusion dialysis apparatus capable of handling large volumes of biological fluids e.g. urine with an efficiency roughly thirty times that of previous methods of dialysis. Furthermore it has been possible to retain a large fraction of some substances of medium molecular weight (above 1000) ordinarily lost in dialysis while at the same time effecting very efficient removal of inorganic salts and smaller interfering compounds such as urea.

This method has made possible the isolation and characterization of two new proteins from urine, and the concentration of antidiuretic and antitryptic factors in urine. Drs. Saroff and Dillard propose to continue the use of this method for the separation and isolation of substances of physiological interest from urine and other biological fluids and tissues. They have obtained the use of electrophoretic equipment for the characterization of these substances and will be assisted in this by Mr. Rosenthal, a junior chemist. Some sedimentation data can be obtained from ultracentrifuges available for occasional use here at the institutes, but an adequate number of runs for the estimation of molecular weights of these proteins will require the availability of an ultracentrifuge for extended periods.

Further characterization of the above substances will be available in the form of amino acid components determined by chromatographic methods by Dr. Irreverre. Particular interest in partial degradation products, as to terminal amino groups, amino acid components, and other unusual components e.g. polysaccharides, pigments complexed with them, will be stressed in this approach to their characterization.

In addition, Drs. Saroff and Dillard propose to develop from the general principle of the membrane diffusion dialyzer a method of continuous electrophoresis of large volumes of biological fluids to provide electrophoretic separation of charged macromolecules on a large scale.

Dr. Saroff is further interested in continuing a long term project centered around the chemical modification of specific chemical groupings in

proteins with its goal the study of the "geography" of protein molecules by means of the activity of certain of these groups. He has initiated a program of progressive methylation of serum albumins with dimethyl sulfate and the study of these modified albumins by infra-red spectroscopy in cooperation with Dr. Scheraga of Cornell University.

Another avenue of approach to the role of macromolecular substances in the biochemistry and physiology of man and animals has been the study of the interactions of these substances in vivo and in vitro. Several simultaneous approaches are being made to this problem.

Drs. Williams and Smith have developed a method of continuous scanning of chromatograms of proteins or other compounds labelled with radioactive isotopes. This device has made possible the development of a method of antigen-antibody titration on paper chromatograms using one hundredth or less of the amounts of antiserum and antigen (labelled with  $I^{131}$  or  $S^{35}$ ) used in previous techniques. Such a technique makes it possible to follow in detail the antibody response of individual small animals on a day to day basis impossible with other methods because of the large amount of serum required.

Considerable time has been devoted to the study of the mechanism of protein movement on paper chromatograms with definite evidence evolving that proteins unlike smaller molecular weight substances move over a thin layer of denatured protein. Successful protein chromatography therefore requires either sufficient protein to provide this layer or pretreatment of the paper to prevent denaturation. Work is in progress on the role of "Tween" substances, organic salt complexes of proteins, and methods of pre-treating paper to provide suitable media for protein paper chromatography.

Along with and somewhat dependent on these studies are projects initiated along the lines of measurement of a wide group of protein complex formations especially those which occur in solution under physiological conditions and are therefore impossible to measure by usual precipitation and isolation techniques. Among these are soluble antigen antibody complexes in the region of antigen excess, combination of antigens with non precipitating ("Univalent") antibodies, normal gamma globulin-albumin complexes, polysaccharide-protein complexes, e.g., dextran serum protein complexes and finally cooperation with another project on metallo-protein complexes.

It is proposed to continue these studies with a view to entering into the characterization, identification of origin, metabolic end products and functional interactions of substances isolated from biological fluids by Drs. Saroff and Dillard as they become available.

Independently it is proposed to apply isotopic tracer and radio chromatographic techniques to protein sensitivity reactions in animals in an attempt to characterize and if possible isolate non-precipitating antibodies of sensitized guinea pigs and rabbits. Further it is proposed to attempt the inhibition in vivo and in vitro of these reactions with partial degradation products produced by enzymatic and other methods of hydrolysis.

Dr. Knox who will be leaving the group this fall has nearly completed one part of a study of the relationship of the size of antigen dose to the rate of antibody formation in animals injected with the same antigen (bovine serum albumin) one year previously. Preliminary results indicate that the rate is a direct function of dose size. His immunological techniques have aided in the characterization of a serum albumin-like substance isolated from urine.

From three separate directions the interests of the group have converged on the study of the nature and function of metallo organic complexes

The studies on chromium phosphate which involved the preparation of the hydrated and anhydrous chromium phosphate the study of which led to some new concepts of the bond energies of the water of crystallization of this compound in a paper by Drs. A. T. Ness, R. E. Smith and Mr. R. L. Evans.

The investigation of particle size in relation to phagocytic rates initiated by Drs. Endicott and Smith led to the preparation and sedimentation data for particles varying from 25 to 1000  $\mu$  in a fairly stable colloidal suspension required for animal injection. Drs. Ness and Irreverre made notable contributions to this with the cooperation of Dr. Merryman of the Naval Medical Research Institute who made the measurements of particle size by electron microscopy.

Control of the starting material and conditions of synthesis of the chromium phosphate led to polarographic studies of chromium complexes with various inorganic and organic ions to be reported by Saroff, Ness and Evans.

Dr. Saroff's studies of zinc and mercury complexes of albumins have nearly reached the publication stage.

Dr. Ness in cooperation with Dr. Brecher has begun the preparation of some gold salts of thio sugars and other thio compounds in an attempt to further clarify the physiological derangement which leads to permanent obesity in mice. The physiological importance of this investigation appears to lie in the permanent alteration in the Krebs cycle of animals made obese by these compounds.



Combinations of these approaches may well lead to better understanding of alterations in enzyme and protein hormone physiology conditioned perhaps by substitution of an abnormal metal for the usual zinc, manganese, or cobalt complexes known to function extensively in enzyme systems.

Dr. Bragdon has continued his study of experimental atherosclerosis in the rabbit with results which have merged his interests in lipo-proteins with those of the rest of the group. In addition to a colorimetric method of blood lipid determination, he has accomplished the transfusion transfer of rabbit atherosclerosis from donors made hypercholesteremic by cholesterol feeding. Also he has noted for the first time the spontaneous occurrence of atherosclerosis in rabbits in certain age periods. He has therefore initiated and plans to continue a program of isolation and characterization by ultracentrifugal methods and other fraction methods for lipo-proteins of the type found in human atherosclerosis.

Drs. Dillard and Irreverre also cooperated with Dr. George Brecher of the Hematology Section of this laboratory and Dr. E. P. Cronkite of the Naval Medical Research Institute in a National Blood Program Project related to the study of radiation damage in animals. They have devised a method for the separation and concentration of apparently unaltered platelets from the blood of humans, dogs, and guinea pigs in yields of 70%, 50%, and 80% respectively. Dog platelet transfusions are being used in current studies of the coagulation defect and bleeding tendency produced by massive x-irradiation.

In addition Dr. Dillard has cooperated with Dr. James H. Peers in studies directed toward the isolation of an encephalitic factor in rabbit brain resulting in concentration of the activity in a fraction representing 1/50 of the starting material. Continuation of these isolation studies is proposed in cooperation with Dr. Peers and with Dr. George Hottle of the Microbiological Institute directed toward the production of rabies vaccine free of the encephalitogenic factor and the characterization of this factor.

Dr. Elijah Adams is assigned to the Laboratory for the Study of Hereditary and Metabolic Disorders, University of Utah College of Medicine. Dr. Adams has attacked the problem of characterization of the proteolytic enzymes of pituitary tissue and of mammalian erythrocytes. He has purified two peptidases, prolidase and tripeptidase from horse erythrocytes and partially purified two proteinases from hog anterior pituitary glands. ACTH contamination with these enzymes leads to inactivation of the ACTH under certain conditions.

A system of large-scale continuous isolation of proteins, polypeptides, polysaccharides from biological fluids was developed.



Results:

Development of a continuous membrane diffusion dialyzer.  
Preliminary experiments on the use of this apparatus as a diffusion rectifier.

Isolation and characterization of two new proteins from urine.

Study of the activity of terminal groups in proteins.

Results:

Methylation of carboxyl groups produces changes in infra-red spectrum suitable for quantitative measurement of these groups.

Chromatographic measurement of protein interactions.

Results:

Development of a continuous scanning device for chromatograms of isotopically labelled proteins.

Chromatographic method of antigen-antibody titration.

Evidence for existence and separation of protein-protein complexes including soluble antigen-antibody complexes and normal gamma globulin-albumin complexes.

Evidence that proteins chromatograph over a layer of denatured protein.

Experimental atherosclerosis in rabbits.

Results:

Colorimetric method of blood lipid determination.

Transfusion transfer of experimental atherosclerosis from hypercholesteremic rabbits.

Occurrence of spontaneous atherosclerosis in rabbits.

Isolation and concentration of platelets for transfusion studies in irradiated animals.

Results:

Isolation and concentration in 50-80 percent yield of apparently unaltered platelets from man, dog, and guinea pig.

Successful transfusion of platelets into dogs.

6. Isolation of encephalitogenic factor from rabbit brain.

Results:

Concentration of the factor in a fraction consisting of 1/50 of starting material.

7. Characterization of the proteolytic enzymes of pituitary tissue and of mammalian erythrocytes.

Results:

Purification of two peptidases - prolidase and tripeptidase from horse erythrocytes to be followed by characterization of these enzymes as well as determination of terminal amino groups by the Sanger technique.

Further purification of two proteinases from anterior hog pituitary gland, contamination of ACTH with these with resultant inactivation of the hormone under certain conditions.

Projects concluded:

1. Studies on phagocytosis in relation to particle size using radioactive chromium phosphate.
  - a. Polarographic behavior of chromium complex ions elucidated.
  - b. Evidence for a new concept of hydration of chromium phosphate crystals.
  - c. Phagocytic studies to be published by Endicott and Smith.
2. Metabolism and Immune Response to  $I^{131}$  labelled bovine serum albumin.
  - a. Rate of antibody formation is a direct function of size re-injected dose in animals injected one year previously.

SECTION ON PATHOLOGIC ANATOMY

This section devotes practically its effort to morphologic studies of diseased and normal human and animal tissues. In order to obtain a supply of human tissues for investigation, diagnostic service on surgical and post mortem specimens is provided a number of Federal institutions, such as hospitals of the Indian Service, Bureau of Prisons and a number of the smaller installations of the Public Health Service. This material and autopsies at St. Elizabeth's Hospital furnish the basis for a training program in pathology for junior medical and veterinary officers and radiology trainees officially approved by the Council on Medical Education and Hospitals of the American Medical Association. The section also undertakes a large volume of morphologic work on experimental animals, both independently and in cooperation with other units of National Institutes of Health.

This Section maintains a tissue processing laboratory for the preparation of microscopic slides by a large variety of routine and special technics. Its facilities are available to and used on occasion by all units of the National Institutes of Health having interest in problems of tissue morphology. During the past year the laboratory processed and the section on Pathologic Anatomy

rendered diagnoses on 2202 surgical specimens, 242 human autopsy specimens and 4596 animal specimens submitted from various sources. This is an over-all increase of 30% above the previous year.

Cortisone and ACTH were found to inhibit markedly the usual inflammatory reaction of the Arthus phenomenon in rabbits. Cortisone greatly exaggerated glycogen storage in the liver while ACTH depleted the lipid storage in the adrenal. Results in press.

In a small series of guinea pigs 12.5 mg/k cortisone had no curative effect on allergic encephalitis produced by injection of brain with Freund adjuvant. Administered with the brain injection and daily for 28 days thereafter it caused some reduction in disease incidence and an apparent abolition of the lymphocytic reaction perivascularly in the brain and about the injection site.

Virus isolated from cases of "herpangina" or vesicular sore throat was shown to be identical pathogenically in mice with the Cocksackie virus. In cooperation with Microbiological Institute the results were published and an exhibit prepared and shown at the annual meeting of the American Medical Association.

In an attempt to confirm the results of Gilman & Gilman, aqueous solution of trypan blue was administered fortnightly to stock rats for 12 months but no tumor-like tissue reactions were obtained.

In cooperation with the Laboratory of Biologics Control and the Laboratory of Physical Biology the development and structure of the Negri body of rabies in inoculated mice was investigated with the electron microscope. The photographs confirmed the traditional appearance of the Negri body, but failed to reveal the presence of elementary virus particles either before or after the formation of the Negri body. Results in press.

In cooperation with the Laboratory of Physical Biology glycogen was demonstrated to be visible in human liver examined by the electron microscope. Both histochemical and morphologic methods were used to establish its identity. Published.

Study of the histochemically demonstrable glycogen in the human heart muscle showed it to be abundant in infancy and therefore of little diagnostic value as well as confusing the traditional picture of glycogen storage disease. It was rarely present in adults save in diabetics, but could not be correlated with the clinical state of the diabetes at death. Results published.

The staining efficiency of various technical modifications of the Schiff reagent were studied, and critical evaluation made of the many empirical formulae published in the literature.

A study of the histochemistry of the retina was completed. Three retinal lipoids appear to be present, a galactolipin-protein complex, and an unsaturated lecithin being probably present in the acromeres, and a saturated phospholipid in the ellipsoids. In press.

A reevaluation of decalcification methods in relation to special histochemical methods of study was completed. In press.

The Bauer chromic acid and Casella permanganate-Schiff methods were shown also to demonstrate 1, 2 glycols, but differ from the periodic acid method in that they further oxidize the aldehydes to a Schiff-negative state. Published.

A modification of the periodic-Schiff procedure was devised which differentiates basement membranes from reticulum and collagen. With the aid of this procedure the reactions of the several types of connective tissue to enzyme digestion, silver impregnation and the like were re-explored. Published.

New methods for the fixation of eyes, for the demonstration of relatively insoluble lipoids, and for the manufacture of the Schiff reagent were devised and submitted for publication.

In cooperation with the Laboratory of Physical Biology it was found that rats exposed to a simulated altitude of 25,000 feet frequently developed a vegetative endocarditis upon which bacteria could be localized by intravenous injection. This furnishes a method for the experimental production of bacterial endocarditis. Results published.

Other cooperative studies have dealt with the pathologic effects of intoxication with "methylal," with the influence of dietary choline upon the production of hepatomas in mice, and with the production of tissue calcification in mice maintained on a low protein diet.

Infectious diseases: In cooperation with the Microbiological Institute the principal work has been with the Coxsackie group of viruses. The standard strain has been successfully propagated in embryonated eggs where, as in suckling mice, it produces widespread destruction of striated muscle. In the chick the infection may practically denude the skeleton of muscle yet the chick continues to live till time of hatching. Also the virus appears to lose some of its previous strict tropism for muscle and striking lesions are produced in the skin and occasionally in the liver. Studies are continuing on the possible modification of the pathogenic properties of the virus by egg passage and the effect of age and other factors on the infection in the chick. Smaller studies are being continued on the comparative pathology of various strains of the Coxsackie virus and on the myocarditis allegedly due to poliomyelitis. Work on the topographic pathology of poliomyelitis in man continues with the examination of the pons and cerebellum in available material.



Allergic encephalitis: Work is continuing on the attempt to localize and, if possible, isolate the factor in brain tissue responsible for the production of allergic encephalitis which closely simulates the encephalitis which occasionally follows vaccination or infectious diseases in man. In cooperation with the Microbiological Institute a large series of chemical fractions and extractives of brain tissue were tested with inconclusive results due to the low content of encephalitogenic factor in the material. A more promising approach is now being made in subjecting brain tissue to extraction with increasing concentrations of salt solution and at different pH in the cold. By this means it has been possible to separate out half of the brain protein, leaving the encephalitogenic factor in the residue without denaturing the proteins. Work will continue along this line in an attempt to separate the antigen from the encephalitogenic factor in rabies vaccine, and to define the properties of the factor.

Histochemistry: The most important projects in this field have been first the development of the performic acid Schiff reaction as a specific color reagent for ethylene linkages and which so far stains selectively ceroid pigments, part of the lipofuscin pigments of the ovary, adrenal and heart and the acromeres of the retinal rods. Secondly it was found that standard hyaluronidase from bull testis still abolishes metachromasia in certain tissue sites after boiling the enzyme, although the in vitro ability to break down hyaluronic acid was destroyed by boiling. Hence a heat-stable enzyme, tentatively designated "chondromucinas" is presumed to be present in certain commercial preparations of hyaluronidase. The metachromasia, previously believed due to hyaluronic acid on the strength of its abolition by hyaluronidase, is now considered to be due to the presence of chondromucin.

If verified this will require revision of much of the work hitherto reported on the localization of hyaluronic acid in tissues. Other problems currently under investigation are determination of the effects of bromination upon Schiff-positive materials, the source of the direct Schiff reaction of substances containing unsaturated compounds, development of more specific staining methods for acid mucopolysaccharides, development of a freezing-drying tissue technic to operate at atmospheric pressure, study of the distribution of glycogen in various tumors, and of granular material present in various types of tissue cells.

Endocrine pathology: Current projects deal with the effect of prolonged administration of cortisone on the morphology of the cells of the anterior lobe of the pituitary and on its ACTH content, with the selective staining and morphologic changes in the hypothalamic-hypophyseal system in cortison-treated dogs with reference to the problem of neuro-secretion, and with selective staining of alpha and beta cells of the pancreas preparatory to experimental studies on carbohydrate metabolism. In addition cooperative assistance is furnished the Section on Endocrinology in examination of experimental animals after brain injury, to the Department of Animal Husbandry of the University of

Maryland in relation to their investigation of ketosis in cows, and to the Public Health Service of the State of Brazil in an investigation of the endocrine system in human cases of exfoliateous pemphigus.

Shock studies: In cooperation with the Section of Pharmacology an extensive study of the histopathology of experimental haemoglobinuric or lower nephron nephrosis produced in mice by burn shock is in progress. The pathologic changes closely approximate those observed in human subjects, and permit a study of the influence of various types of shock therapy on the renal lesions. Saline therapy appears to have little effect on the development of nephrosis, while dextran reduces the quantity of hemoglobin casts but produces a diffuse dilatation of renal tubules believed to be due to the presence of accumulated dextran. Studies on the effect of polyvinyl pyrrolidone (PVP), plasma and gelatin are in progress. In the course of the study a method, based upon the performance of the periodic acid Schiff reaction in alcoholic rather than in aqueous solution, was devised for the histochemical demonstration of dextran in tissue sections and study of its distribution and removal are continuing. As a by-product of this investigation it was found that fasting 12-24 hours alone was sufficient to produce fatty metamorphosis of the normally fat-free renal tubules of the female Swiss mice.

Toxicology and physical agents: Studies are continuing on the effect of various agents such as methionine on the histologic changes induced by poisoning with various halogenated hydrocarbons. Exposure of rats to reduced atmospheric pressure corresponding to an altitude of 25,000 feet has resulted in the production of a vegetative endocarditis and other degenerative changes. Bacterial endocarditis can be superimposed on such hearts by intravenous injection of pathogenic bacteria, and studies are in progress to test the efficacy of various therapeutic measures on this experimental endocarditis. Attempts are being made to shorten the acclimatization period necessary before injecting the streptococci and to determine the influence of cortisone and dextran on the endocarditis. Other simulated altitude studies include the effects of intermittent exposures, the effects of long rest periods and the effects of irradiation on the acclimatization of rats. A study on the acclimatization of turtles to high altitudes is under way.

#### SECTION ON PHARMACOLOGY AND TOXICOLOGY

Traumatic shock: Investigations into the mechanisms and therapy of burn and tourniquet shock and hemorrhage in mice, begun ten years ago, were continued. One of the problems concerns the criteria used for comparing the value of a given therapy. As our studies have been based on mortality, a comparison of the criteria commonly used in the clinic was made against mortality.

Plasma and serum albumin therapy restored bleeding volume (blood volume) and hematocrit (blood concentration) to normal, but the effects were transitory. Oral saline therapy had very little early effect, although restoration

gradually occurred over twenty-four hours. Thus, the acute response of the circulation to therapy may not be a reliable index of effectiveness of therapy.

It was also shown that most of the therapeutically administered fluid, whether plasma or saline, can be recovered in the injured areas two hours after injection. This local accumulation in the injured areas explains the need for large volumes of saline in therapy, and possibly why large amounts of sodium and fluid are retained in the body during the period of wound shock.

Another project dealt with the comparative efficacy of some plasma substitutes in the treatment of shock.

Comparison of dextran and polyvinyl pyraolidone (P.V.P.) with saline in single doses indicated that the sodium content of these plasma substitutes was an important part of their effectiveness in preventing death from shock. In burn shock some evidence of toxicity was shown, not manifest in normal mice. In cooperation with the Laboratory of Pathology, histological kidney changes were demonstrated; their significance has not been established.

In cooperation with Division of Research Grants a clinical project on evaluation of saline therapy in burn shock was set up in Lima, Peru. Similar projects were also begun in New York City and Galveston, Texas.

Histamine metabolism: The mechanism of breakdown of histamine by the enzyme diamine oxidase was further elucidated, and the end products isolated. The enzyme which was shown in this laboratory to acetylate histamine (thereby destroying its biological activity) was further purified and the mechanism of its action was shown to involve Coenzyme A. Its relation to other biological acetylations is under study.

Physiology and pharmacology of nerve conduction: The effects of anoxia, of cocaine, veratrine, and iodoacetate on the electrical phenomena of nerve conduction were correlated with the sodium-potassium exchange between the nerve and its environment. The evidence indicates the importance of potassium in the transmission of nerve impulses, and that the effect of drugs on nerve conduction are concerned with these changes.

Bacterial chemotherapy: A number of new derivatives were synthesized for testing against tuberculosis. These were related to diaminodiphenylsulfone (DDS) or to the aldehydethiosemicarbazones, parent compounds that possess activity. The thiosemicarbazones, although active in vitro, were toxic in animal experiments. Several sulfone derivatives were active in vitro and require further toxicity and therapeutic tests.

Further studies in tuberculosis include the pharmacology of various sulfone drugs used in tuberculosis and leprosy. One compound, the hydroxy ethyl



derivative (HES), is not changed in the body to the parent D.D.S. It is given a clinical trial with favorable preliminary results.

The metabolism of the tubercle bacillus in relation to sources of nitrogen in the culture medium is under study.

Further work on the purification of *Nocardia* was carried out. While partial purification was accomplished by extraction with solvents and adsorption on resins, insufficient material was obtained for therapeutic tests.

A program of testing drugs against rat and mouse leprosy is being set up in cooperation with the American Leprosy Foundation.

Toxicology: A water soluble metabolite excreted in the urine after chlordane administration is under investigation for chemical identity.

#### LABORATORY OF PHYSICAL BIOLOGY

Paul A. Neal, Chief

The Laboratory of Physical Biology is concerned with the study of physical phenomena associated with biological systems. The current research of the Laboratory deals with studies of the influence of physical factors (such as high and low energy radiation, light, heat and pressure) upon cellular activity and with the fine structure of cells and tissues and their components, for example, viruses, muscle fibriles and connective tissue. Such studies are designed to give an understanding of the macromolecular components of living matter and to interpret the physico-chemical mechanisms which underlie the metabolism of both healthy and diseased cells.

#### SECTION ON MOLECULAR BIOPHYSICS

The work of this section continues those listed in last year's report together with a renewal of an earlier study of the fine structure of cellulose. The systematic investigation of membranes and membrane model systems was continued. The theory of mosaic membranes composed of ideally cation selective and ideally anion selective parts was tested, and confirmed quantitatively in systems which do not involve the use of auxiliary electrodes. It was demonstrated that anomalous osmosis may occur in systems in which all solutions involved are at the physiological concentration level. A method was developed for the preparation of strong acid type permselective collodion base membranes.

The projects under way in this section for the last year involved:

Mode of growth and multiplication of plant, animal and insect viruses in sectioned tissues. This study has resulted in the visualization of elementary



virus particles developing in cells infected with: influenza, vaccine virus, the virus of Newcastle disease, rabies, several kinds of insect virus.

Mode of growth and multiplication of bacteriophages in bacterial cultures. The discovery has been made in this study of additional forms which probably represent particles of bacteriophage in course of development.

Visualization with electron microscope of lethal action of various radiation on micro-organisms. This study has resulted in the discovery that especially high yields of bacteriophage can be obtained from X-ray "killed" bacteria.

Radio-phosphorus metabolism of bacteriophage-diseased bacteria. This study has demonstrated radically different metabolic processes in bacteria diseased with different strains of bacteriophage.

Macromolecular structure of sectioned tendon, nerve and multiplying cells. These studies have resulted in the visualization of connective tissue fibres forming from solutions of collagen and determination of the physico-chemical conditions of their formation.

The electron microscopy of cellulose structures as elaborated by a variety of plant tissues. This has resulted in the preparation and electron microscopy of thin sections of wood showing the arrangement of its cellulosic fibres.

Continued collaboration with the National Institute of Dental Research in studies of the macromolecular texture of teeth. These studies have produced the first electron micrographs showing the development of enamel in embryonic teeth.

Strong acid type permselective collodion base membranes. Such membranes promise to become an important research tool, being free of certain shortcomings of the previously available weak acid type perm-selective collodion membranes.

Anomalous osmosis in vitro under conditions which are analogous to certain physiological situations. It was demonstrated that anomalous osmosis may occur in systems in which all solutions involved are at the physiological concentration level and that the liquid transportation rates obtainable under such conditions are considerably in excess of any of those reported for the liquid movement across living membrane in the intestine.

Mosaic membranes composed of ideally cation selective and ideally anion selective parts. The theory of these membranes was tested and confirmed quantitatively in systems which do not involve the use of auxiliary electrodes. The main significance of this is that numerous complex phenomena occurring across

membranes in living organisms can now be discussed with a much higher degree of confidence in the correctness of involved and detailed theoretical deductions than was possible heretofore.

#### SECTION ON LOW ENERGY RADIATION BIOLOGY

The first study on photosynthesis and respiration in *Chlorella* has been completed and papers are in progress of preparation. The findings are of special interest at this time of heated controversy. They are briefly:

The number of quanta required for photosynthesis of one molecule varies chiefly from 6 to 8 and rarely as high as 12. No support was found for values of 4 or lower.

There is no evidence for changing efficiency with intensity up to 10 times respirational compensation.

Respiration changes are found resulting from illumination by often as much as fourfold. These changes show time delays from about a minute to several hours.

These respirational changes make short period manometric observations subject to large possible errors depending upon the conditions of the experiments. The sensationally low quantum values recently publicized (1 to 4 quanta/molecule) may be explained by this observation.

The study of aerosols, completed and published in two papers, has been well received and new projects along these lines are under consideration in connection with defense activities.

The development of infra-red facilities has progressed. A novel servo mechanism double beam modification has been put into preliminary operation. Personnel is being trained and gaining experience in the operation of conventional equipment.

Papers on photoisomerization of ergosterol, vitamin D and related molecules are in progress of preparation.

#### SECTION ON NUCLEAR RADIATION BIOLOGY

The regular planned work of this section has been somewhat curtailed because of some research projects undertaken at the request of various agencies in the National Military Establishment. Some of these projects are classified but all are considered to be of importance to the National Defense and this section was requested to undertake them because of special competencies in personnel and equipment.

One new project, essentially completed, was an evaluation of antibiotics as therapeutic agents against radiation injury. Some antibiotics were shown to actually increase the mortality from radiation in mice and even the most promising was erratic in action and in some groups failed to provide protection. This is a finding of considerable importance to defense planning against atomic weapons since antibiotics are at present the only therapeutic agents with any promise for large-scale use. Detailed studies of the mechanism of action of these compounds are being planned.

Single celled animals put into irradiated water have shown injuries comparable to those seen when the animals themselves are irradiated with much larger doses. This effect is enhanced when the water is irradiated in the presence of air. This important finding will be investigated in the hope of shedding light on the role of body water and oxygen tension in radiation injury.

Radio-chemical analyses of eye tissues showed that neutron-induced cataracts are not formed because of the presence of elements having a high capture cross section for neutrons. This implies that these cataracts are produced essentially by fast, rather than by slow, neutrons.

A study of the neutron-induced radioactivities in some common foods has shown that food in containers undamaged by an atomic blast will be safe for human consumption.

Studies of the effects of radiation on the enzyme systems in the spleen have been started and the preliminary results indicate some rather profound effects.

Work is continuing on the physical properties of the reaction products formed from macromolecules by X-rays. Size and shape factor determinations made from viscosity, electrophoretic and ultracentrifuge measurements show that some macromolecules are fragmented by radiation while others polymerize into still larger molecules.

All of the important radiation products from glycine and alanine have been identified and quantitatively determined and this work is being extended to the polypeptides. Work with radioactive tracers will determine the point of origin of some of the breakdown products and will permit a considerable insight into the mechanisms of action of radiation on various chemical bonds.

#### SECTION ON PHYSIOLOGICAL PHYSICS

Acclimatization of rats and dogs to high altitude oxygen lack has shown that reversible changes at tolerable levels can be followed quantitatively. Removal of stress results in regained function although persistent histopathology may result in some systems. Generally, germinal tissues and kidney functions are reversibly affected while certain heart and kidney lesions are

persistent. Studies of hemo-dilution with dextran have been started to observe effects of opposed factors to those of altitude-induced polycythemia. The observation of heart lesions due to hypoxia has led to a study of experimental endocarditis and the initiation of a cooperative study of the efficacy of certain antibiotics.

The study of enzymatic reactions of muscle proteins and fibrinogen in polymerization has yielded a new biophysical approach to such basic physiological phenomenon as energy exchange and structural conversions believed to be fundamental in all living material.

The use of respiratory analysis through nitrogen transfer from lungs to blood has aided in demonstrating the different efficiencies of various resuscitative methods and in comparing them to that of free breathing.

Studies in insect respiration, particularly of the relation between cuticular and spiracular paths, have shown certain theoretical considerations of gas diffusion in small biological vessels and orifices to provide properties adapting the organisms to efficient function. Theoretical considerations indicate a possible cyclic flow of gases, both into and out of the tracheae.



NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES  
AND BLINDNESS

Pearce Bailey, Director

One of the two new institutes established by Congress in the National Institutes of Health in 1950, the National Institute of Neurological Diseases and Blindness began to shape up organizationally. Because no funds and staff were available for the operation of the new institute during fiscal year 1951, the Surgeon General designated the National Institute of Mental Health to administer the program. With the appointment of Dr. Seymour S. Kety as Scientific Director of basic research which will be conducted jointly by the National Institute of Mental Health and the National Institute of Neurological Diseases and Blindness, planning for the intra-mural research program of the two institutes got underway. For fiscal year 1952, funds are being appropriated for the program of the new institute, and its director and key staff will be appointed.

Research projects in neurology and blindness which were receiving support from the National Institute of Mental Health and the Division of Research Grants of the National Institutes of Health were transferred to the jurisdiction of the newly-authorized Institute. These studies included research in neurophysiology, neuropathology, neurosurgery, neuroanatomy, specific neurological diseases, vision and other special senses. During fiscal year 1951, fifty such projects received support in the amount of \$491,222, as compared to \$287,221 spent during the preceding year in support of these areas of study.

The National Institute of Mental Health continued to support teaching grants and training stipends in neurology. During the year, seven grants were made totaling \$77,881 for the support and development of the teaching of neurology. Of this amount, \$55,681 was granted to seven medical schools to promote graduate neurological teaching, and \$22,200 to assist eight physicians seeking specialized training in neurology.

The twelve members of the National Advisory Neurological Diseases and Blindness Council were appointed by the Surgeon General, and the Council met three times during the year. At the first meeting, in October 1950, the Council was concerned with organization. At the second meeting, in February 1951, the Council reviewed 46 research proposals, of which 25 were approved for support totaling \$235,901. At the June 1951 meeting, the Council considered 64 research applications and approved 47 research projects for support in the amount of \$374,292.

Most of the projects were approved to begin in 1952 when the Institute received its first appropriation of funds. These projects involve among other subjects, multiple sclerosis; the physiological pathogenesis of epilepsy; growth and function of the nervous system; investigations on the ultrastructure and chemistry of nerve; basic studies on cerebral palsy; the reproductions of retrolental fibroplasia in newborn and premature animals; physiology of the visual system; the physiopathology of the cornea and corneal grafts; prevention of experimental allergic encephalitis; study of the peripheral neuromuscular system in man, correlating the electrical activity and muscle tension; development of the human visual pathways.

DIVISION OF RESEARCH GRANTS

Ernest M. Allen, Chief

C C N T E N T S	Page
General statement: research grants.....	213
Significant contributions in the grant program.....	214
General statement: research fellowships.....	218
Symposia.....	218
National Advisory Councils.....	218





DIVISION OF RESEARCH GRANTS

Ernest M. Allen, Chief

RESEARCH GRANTS: GENERAL STATEMENT

The purpose of the Public Health Service Research Grants Program is to support research in medical and allied fields for which funds are not adequate or which could not otherwise be conducted in the grantee institution. The major objectives of the grants program are (1) to expand research activities in universities and other institutions, (2) to stimulate the initiation of research in small colleges where previous research programs have been very limited or non-existent, (3) to encourage investigators to undertake research in neglected areas, and (4) to provide training for scientific personnel.

In carrying out these objectives, the aim of the Public Health Service is to promote the highest quality of endeavors in both fundamental and applied research and render assistance to public authorities, scientific institutions, and scientists in these endeavors.

Grants may be made in support of research work conducted outside the territorial limits of the United States under the auspices of foreign organizations where satisfactory evidence is provided that (1) the research is of such nature that for scientific reasons it can best be conducted outside the United States and is of general importance to medical science; and/or (2) the applicant is so extremely well qualified as to specified training, experience, and talents that support of his work is indicated in anticipation of findings of specified importance to medical research. Any research in the field of medicine or research related to or of potential significance to the field of medicine is eligible for support.

Applications for research grants are submitted to the Division of Research Grants for the fiscal administration of all research grant programs and for certain technical services to the administrative staffs of the Institutes authorized to award research grants in the Public Health Service.

Applications for research grants in support of work in cancer are referred to the National Advisory Cancer Council for recommendations to the Surgeon General; those in mental health and closely related fields, to the National Advisory Mental Health Council; those in dental research and closely related fields, to the National Advisory Dental Research Council; those in heart research and closely related fields, to the National Advisory Heart Council; those in arthritis and metabolic diseases and closely related fields, to the National Advisory Arthritis and Metabolic Diseases Council; those in neurological diseases and blindness and closely related fields, to the National Advisory Neurological Diseases and Blindness Council; and those in all other

research fields, to the National Advisory Health Council. Each of the Councils meets three times each year.

During the fiscal year 1951, a grand total of 1724 applications were approved by the Surgeon General in the amount of \$17,304,529 (exceeds appropriation by \$591,529 - made possible through funds reclaimed from grants approved). Of this total, 595 applications for research grants in the non-categorical fields were approved for payment in the amount of \$5,513,617.

The 1724 grants approved for payment in fiscal year 1951 are distributed among 278 institutions located in 44 states, the District of Columbia, 1 territory, and 14 foreign countries. The only states which received no research grants from fiscal year 1951 funds are New Mexico, Nevada, North Dakota, and Wyoming; however, Wyoming had a project which was paid from fiscal year 1949 funds and New Mexico had a project which was paid from fiscal year 1950 funds. In addition to the 1724 grants which were approved for payment from fiscal year 1951 funds by the Surgeon General, there were an additional 89 grants which had been recommended for approval by the National Advisory Councils to the Surgeon General for which there were no funds available for payment.

The vast volume of requests for research grant funds has required the establishment of a priority system for determining payment of a grant application which has been recommended for approval by the appropriate Advisory Council. The priority system was established in order to permit new applications to compete for the scarce funds on an equal basis and to insure a quicker response to the applicant of the action on his request. Attached is a table showing the number of research grant applications approved and amounts approved by the Advisory Councils and the Surgeon General at the three fiscal year 1951 Council meetings. These data, of course, cannot be compared with the fiscal data quoted above.

#### SIGNIFICANT CONTRIBUTIONS IN THE GRANT PROGRAM

##### Cardiovascular

With respect to continuation projects, it is to be noted that the large number of projects activated in the fall of 1949, with the then newly available Heart funds, are now beginning to bear visible fruit; and substantial results have been reported from laboratories in the medical schools of the University of Washington, Baylor University, University of Pennsylvania, University of Illinois and Cornell University, to mention but a few.

A new venture of general importance, launched under a joint recommendation of the Cardiovascular Study Section and the National Advisory Heart Council, consists of the evaluation of ACTH and cortisone in the treatment of rheumatic fever. In this project, seven participating research centers in the United States and Canada and six in England, coordinated by the American

Council on Rheumatic Fever and the Medical Research Council, respectively, and with drugs furnished gratis by the manufacturers, are engaged in a cooperative attempt to determine the value of these drugs in the therapy of an acute attack of the disease, with particular reference to the possibility of preventing permanent cardiac damage. The study is now well under way, and its short-term results are expected to be available within a year. Financial provision has already been provided, however, for several additional years of follow-up. The funds were provided from the special ACTH-cortisone appropriation.

#### Microbiology and Immunology; Virus and Rickettsial

The greatest single impact felt was the shift of a very high proportion of the projects sponsored by these two Sections to the Grant Program of the National Microbiological Institute. The effect of this shift is not evident as yet; therefore, few trends have been discerned. There is the continuing trend of an increase in the number of projects proposed to the two Sections. The Microbiology and Immunology Study Section went on record to assert its feelings that basic research activity was still most important; that it was unwilling to encourage any shift away from this approach toward the practical or defense priority projects at this time.

#### Physiology

The emphasis of applications is shifting with the establishment of the National Institute of Neurological Diseases and Blindness to field of neurophysiology and sensory diseases. Approximately one-third of the requests are now in these fields. A new study section to handle applications in the field of sensory disorders is to be established in the fall of 1951.

#### Surgery

Outstanding accomplishments were made in the following fields through research grants recommended by the Surgery Study Section:

The development of a mechanical heart and lung apparatus to replace the functions of the human heart and lungs for periods sufficient to permit operations within the heart in a bloodless field.

This type of apparatus has now been developed to the point where it is possible to completely by-pass the heart and lungs in an experimental animal for more than one hour and where the major part of the circulation can be carried up to four hours with no apparent ill effects.

Advances have also been made in the development of pumps which will replace the heart while allowing the blood to flow through the animal's own lungs for oxygenation.

Direct surgical operations for the correction of heart defects. In the correction of heart abnormalities, particularly mitral stenosis, important advances have been made.

Advances in skin grafting and wound healing are reported and a project that may lead to important developments in bone grafting has been started.

Important advances were made in the treatment of metabolic disturbances in surgical patients. In the treatment of shock due to burns, extensive research is being conducted.

### Pharmacology

Research dealing with the fundamental nature of drugs is prevalent. Study of chemical constitution and the mechanism of drug action on cellular functions using tracer procedures has been prominent.

Among the outstanding investigations are those concerned with analgesics. These included development of methods especially sensitive and specific for the identification and quantitative measurement of morphine and similar drugs, present in small amounts in body tissues following therapeutic dosage. This makes possible the study of the effect of morphine and its derivatives on intermediary metabolism.

The use of isotopes also makes it possible to study the metabolic fate of barbiturates, about which practically no information is available. Similar studies are concerned with the action of diphenylhydantoin and trimethadione used for the treatment of epilepsy. Knowledge gained from these investigations can lead to the synthesis of better drugs and improvement in the treatment following poisoning with these drugs.

### Morphology and Genetics

Among the outstanding projects in this field are those dealing with cell chemistry and physiology. The relation between cell structure and cell chemistry is being investigated by isolating cellular constituents and determining their biochemical properties. Altered relationships between the nucleus and the cytoplasm of the cell are also being studied. Knowledge of the biophysical and biochemical properties of tissues is being extended by the development of new and improved microchemical and microphysical technics and their application to tissue analysis.

### Tropical Medicine

Important work constitutes a search for and development of new chemotherapeutic agents active against amebiasis, malaria, schistosomiasis, and onchocerciasis.



In spite of advances made in the treatment of malaria during World War II, this continues to be the most important disease in tropical areas. Significant progress has been made in the development of more effective methods of suppression and treatment of malaria, and it is hoped that further work will result in the development of a true prophylactic for prevention of this disease.

Investigations on the biology and chemotherapy of primate malaria, utilizing as a test object Plasmodium cynomolgi infections in the rhesus monkey has yielded information of considerable importance, and furnishes a necessary foundation for the studies of Alving and others on human volunteers.

Additional studies are adding considerably to our knowledge of the pathology, transmission, and control of the disease onchocerciasis.

### Environmental Health

Investigations are continuing in order to develop more economic and scientific methods for bacteriological, chemical and physical analyses in the fields of water supply and sewage disposal.

Studies have been instigated relative to industrial waste problems since in recent years there have been steadily increasing technological developments and changes in manufacturing processes which have created new industrial wastes.

Studies are progressing in the field of milk and food sanitation. The expansion of the frozen food industry has created this necessity for basic scientific knowledge of the bacteriological aspects of frozen food supplies.

Studies are being made on sewage contaminated waters which are used for irrigation purposes in order to determine to what extent, if any, vegetables may become contaminated with pathogenic organisms.

Two studies are in progress on industrial air hygiene, one on asbestos and the other on chromate dusts in relation to possible health hazards, such as lung tumors, which such dusts may create in industrial workers.

### Biochemistry and Nutrition

These projects are increasing due primarily to the interest in normal function which must be determined in the cell before disease function is possible. Fundamental, normal, and abnormal processes are now being studied leading to the healthy and diseased conditions respectively. Such studies are: "Nutritional Factors in the Origin and Growth of Tumors", "Nutritive Requirements of Microorganisms", "Studies on the biochemical Basis for the Development of Resistance by Mouse Leukemias to Folic Acid Antagonists", "Studies to

Increase the Efficiency of Cation Exchangers in Removing Sodium via the Gastro-intestinal Tract", etc.

#### RESEARCH FELLOWSHIPS: GENERAL STATEMENT

Research Fellowships are awarded by the Public Health Service for the purpose of developing competent research workers in the medical and related sciences. These fellowships may be awarded to applicants holding the Bachelor's Degree, Master's Degree (or the equivalent in graduate training), or Doctor's Degree. The funds for payment of the fellowships are appropriated to the categorical institutes from which the Office of Fellowships in the Division of Research Grants draws to pay the stipends. In addition to the stipend, research grants of not more than \$500, plus eight per cent overhead, may be made to non-governmental institutions in behalf of a Postdoctoral Fellow. These funds are intended to reimburse the institution for a part of the expense incident to sponsorship of the Postdoctoral Research Fellow and may be spent in accordance with local institution policy and rules.

During the fiscal year 1951, a total of 1170 applications for fellowships were received. The rate of approvals of the applications for 1951 shows 549 fellowships awarded as contrasted with 527 fellowships awarded in 1950. Of the 549 awards in fiscal year 1951, 225 were predoctorate fellowships, 296 were postdoctorate fellowships, and 28 were special fellowships.

At the present time there are 442 Fellows receiving support from the National Institutes of Health. These Fellows are located at 71 institutions distributed throughout 32 states, the District of Columbia, and 8 foreign countries.

#### SYMPOSIA

Two symposia were held during fiscal year 1951:

<u>Study Section</u>	<u>Title</u>
Experimental Therapeutics	Recent Advances in the Study of Venereal Diseases (4/24/51-4/25/51)
Surgery	Metabolic Disturbances during Surgical Care (1/12/51)

#### NATIONAL ADVISORY COUNCILS

The National Advisory Councils met during the months of October, February, and June. In February 1951 the Councils met in joint session, at which time recommendations were made on the following:

## Manpower Mobilization

The Universal Military Training and Service Bill now before Congress does not adequately provide for the maintenance of the flow of highly trained manpower essential to National defense.

The proposal to select up to 75,000 men per year for college training until June 30, 1954, will result in output below the minimum number of scientists and allied personnel required to sustain the National economy and the defense effort.

Since testimony related to extension of the Selective Service Act and the adoption of universal military service indicates that the armed forces will not induct all fit 18 year olds for two and perhaps three years in order to meet troop strength requirements, we urge that provision be made through deferment for specific fields of study or through selection of men after induction for entrance of 150,000 fit males 18 years of age and older into college in each year to sustain current levels of output in the physical and natural sciences and engineering and to sustain levels of output in other fields at half of current levels.

That board established by the President for the selection of inductees to be sent to college after basic training should contain representation from the medical and health fields.

Since there furthermore can be no firm assurance that the 27 month period of training and service provided by the bill will not have to be increased, we urge that there be no termination date on the authority to select inductees and place them on inactive status for education purposes.

Since it will be impossible to select an entering class of college students for the fall of 1951 under procedures set up under a universal military training and service system, we recommend that special procedures be established to select fit male students to enter college in the fall of 1951.

We recommend that all fit males 18 years of age and older who will be college seniors and juniors in good standing in the fall of 1951 be permitted to complete their college training, that those who will be sophomores in the fall of 1951 be selected by the same procedure that will be applied to those who will be selected to enter college in the fall of 1951, and that the regulations necessary to carry out this policy be promulgated by March 1.

Unless these steps are taken, there will be a sharp drop in the output of college trained people with critically important skills during the dangerous years 1952 and 1953.

We recommend that the Department of Defense promulgate reserve policies which will clarify the status of thousands of scientists whose effectiveness is now impaired by uncertainty.

#### Research Grants policy in the National Emergency

It was recommended to the Surgeon General that the research grant policy continue to be the stimulation of research on the most important problems in medicine and its allied sciences and its support of the best research proposed. The Councils feel that the continuity of basic research is the foundation and the first requirement for all applied research necessary to maintain the national health in times of emergency.



NUMBER OF RESEARCH GRANT APPLICATIONS APPROVED AND AMOUNTS APPROVED  
BY THE ADVISORY COUNCILS AND THE SURGEON GENERAL  
AT THE THREE FISCAL YEAR 1951 COUNCIL MEETINGS,  
NAMELY, OCTOBER 1950, FEBRUARY 1951, AND JUNE 1951

D. of R.G  
8-23-51  
R.P.M.

I N S T I T U T E S	C O N S I D E R E D		A P P R O V A L B Y A D V I S O R Y C O U N C I L S		A P P R O V A L B Y T H E S U R G E O N G E N E R A L	
	N U M B E R	A M O U N T R E Q U E S T E D	N U M B E R	A M O U N T A P P R O V E D	N U M B E R	A M O U N T A P P R O V E D
TOTAL ALL INSTITUTES	2,283 <u>1/</u>	\$26,485,171	1721	\$17,770,475	1632	\$17,144,857
ARTHRITIS AND METABOLIC DISEASES	65	1,018,800	44	533,656	44	533,656
NEUROLOGICAL DISEASES AND BLINDNESS	118	1,203,871	82	713,986	82	713,986
CANCER	408	4,943,169	345	3,789,030	344	3,786,626
DENTAL	49	425,052	37	259,059	35	225,134
MICROBIOLOGICAL	199	1,869,739	174	1,559,481	168	1,522,463
HEART	528	6,627,147	419	5,227,513	403	5,117,869
MENTAL HEALTH	140	2,239,442	78	875,323	78	875,323
DIVISION OF RESEARCH GRANTS	776	8,157,951	542	4,812,427	478	4,369,800

1/ Applications reviewed at more than one council meeting during fiscal year 1951 are tabulated in this table to reflect only the action of the council which last considered the application.



RESEARCH FACILITIES PLANNING BRANCH

C O N T E N T S	Page
Rehabilitation services in the Clinical Center.....	225
Activities of Nursing Consultants.....	226
Legal Aspects of Clinical Center Operation.....	226
Relationships with Professional Associations.....	226
Activities of Organizations and Methods Examiner.....	227
Social Services in the Clinical Center.....	227
Engineering Planning Activities.....	228





RESEARCH FACILITIES PLANNING BRANCH  
FOR FISCAL YEAR 1951

The Research Facilities Planning Branch is charged with the functions of (1) advising the Director of the National Institutes of Health and preparing special studies for the Research Facilities Planning Committee on various plans and problems involved in the construction of the Clinical Center Project; (2) acting in the capacity of liaison with the Public Buildings Service and other groups concerned with constructing and equipping the expansion of these research facilities; and (3) furnishing professional assistance to the Director of the Clinical Center with regard to staffing plans, budgetary requirements, organizational procedures and the procurement of equipment for the Clinical Center.

In addition to its specialized hospital administration, nursing, engineering, organization and methods, and equipment planning personnel, the Branch calls on the services of the Laboratory, Radiation, Dental and Animal Facilities Subcommittees of the parent Research Facilities Planning Committee. Consultants are also engaged for varying periods to assist in specialized problems.

During the fiscal year construction on the Clinical Center had advanced to 59 percent of completion and contracts were awarded for the construction of the Boiler House and the Shops, Laundry and Storage Buildings. A contract for the tunnel piping was also let and work was started on the electrical substation to be located on the National Institutes of Health grounds. Final drawings and specifications were completed for the Animal Building and plans had advanced to near-final stages for the Grounds Maintenance and Chemical Storage Buildings. Functional plans for the Clinical Center's 1,100 laboratory modules had been completed and sent to the Public Buildings Service.

Concerted efforts are being devoted within the present year to acquire key staff for the Clinical Center, develop policies and organizational methods consistent with the Institute structure of the National Institutes of Health, and complete the selection of and contract for those equipment items required to place the Clinical Center in operation.

Rehabilitation services in the Clinical Center. --

The Clinical Center, in its role as a medical research center of nationwide importance for the study of the major chronic diseases, will afford unique opportunities for testing and demonstrating effective treatment methods that have as their goal restoration of handicapped persons to their maximum functioning. Preliminary planning for a comprehensive program of rehabilitation services has been started, based on an integrated approach of such disciplines as physical medicine, psychological and vocational counseling, occupational and physical therapy and related services. Plans for related services to patients in the fields of recreation, bibliotherapy and religious ministry are also being developed.

Legal aspects of Clinical Center operation. --

Detailed study has been made of the present legislative basis for Clinical Center operations in the area of patient care and of needs for additional legislation. Such study has included consideration of such problems as (1) methods of preserving the research function of the Clinical Center, (2) scope of medical services, (3) payment for care, (4) custody and detention of the mentally ill, (5) provision of transportation and care in nursing or convalescent homes, and (6) defense of negligence and malpractice actions.

Relationships with professional associations. --

Close contacts have been maintained with the American Association of Medical Social Workers, the American Association of Psychiatric Social Workers, the American Association of Social Workers, the Social Work Research Group and the Hospital Section of the American Recreation Society. The annual conferences of the American Orthopsychiatric Association and the National Conference of Social Work were attended.

Activities of nursing consultants. --

During the past year the Nurse Consultants of the Research Facilities Planning Branch have been active in (1) development of a pattern of organization for the Nursing Service of the Clinical Center; (2) determination of staffing needs for the opening, and consequent phases of Clinical Center operations; (3) preparation of job descriptions for professional and non-professional nursing personnel; (4) preparation of budget estimates for Clinical Center operations, Phase I; (5) planning for the sequence and timing of activation of nursing units; (6) listing of fixed and movable equipment for nursing areas; (7) planning for the interior decoration and furnishing of the Clinical Center; (8) preparation of the basic outline for the Role of the National Institutes of Health in an Emergency Plan for Civil Defense; (9) preparation for the cornerstone and open house activities; and (10) interviewing prospective applicants for Clinical Center nursing positions.

Other activities of the Nurse Consultants included (1) conferences and interviews with student nurses, nursing educators, nursing leaders from foreign lands, and other interested visitors, to acquaint them with the activities of the National Institutes of Health and current planning for the Clinical Center; (2) observation visits made and reports compiled on the nursing services in five representative psychiatric hospitals, (3) participation in the program of the State and Territorial Health Officers Annual meeting; (4) conduction of a two-week Institute on Attendant Training, at the request of the Massachusetts Department of Mental Health; and (5) the completion of a Study of Staffing Patterns in Seven Research Units, as a joint project with the Division of Nursing Resources.

During the coming year, with the appointment of key nursing personnel, RFPB nursing activities will move from a consultative to an operating phase, with activities centering around (1) intensification of recruitment activities, (2) acquisition of expendible equipment, (3) formulation of policies for the administration of nursing services and (4) the development of procedures for nursing care.

Activities of organizations and methods examiner. --

Emphasis in planning for operation of the Clinical Center has been placed upon two major areas: (1) identification of the social, emotional and psychological needs of patients participating in clinical investigation, together with development of plans for organization of the Clinical Center departments of social service and of rehabilitation; and (2) clarification, in cooperation with the Office of the General Counsel, of the legal aspects of patient care in Clinical Center operations.

The last half of the fiscal year has been spent in a full-time detail, as executive secretary, to the PHS Committee on Rehabilitation. This committee, appointed by the Surgeon General and composed of representatives from each bureau and from the Office of Vocational Rehabilitation, is charged with responsibility for appraising the current rehabilitation programs of the Service and for developing recommendations for their improvement and expansion. The 7-member committee is assisted by a 10-member task force which constitutes the active study group responsible for formulation of proposals. The report of the committee, it is expected, will constitute a "white paper" on rehabilitation, and will include long-range goals for program planning and fiscal policies.

Social services in the Clinical Center

Early in the year proposals for the organization, functions and staffing of the social service department in the Clinical Center were completed, following extensive consultation with leading practitioners in the fields of medical social work, psychiatric social work and social group work, with schools of social work and with heads of social service departments in a large number of hospitals. These proposals are now being discussed with the Institute Directors and, where possible, their clinical investigation chiefs.

Planning has been predicated on the recognition that social and psychological factors are of importance in studying causation and treatment of the diseases to be investigated in the Clinical Center and that, in many areas, the social sciences can make significant contributions to medical research, and that social work, as a discipline based on the integration of several social sciences, can provide useful adjunctive services to medicine.



Engineering planning activities. --

The Engineering Planning activities of the Branch were chiefly directed toward preparing basic engineering and other data for the Public Buildings Service's design of the Clinical Center. Engineering consultants in the Branch provided various technical assistance to the Research Facilities Planning Committee and its Laboratory, Radiation, Dental and Animal Facilities Planning Subcommittees on special requirements for the Clinical Center project.

Major activities around which the engineering planning functions were centered included: (1) assistance in developing functional plans and reviewing final plans for the Boiler House, Shops, Laundry and Storage Building, Animal Building and other buildings; (2) developing mechanized, labor-saving equipment for various central facilities; (3) conducting pilot plant studies on various special laboratory equipment; (4) planning typical laboratory layouts and assisting in the selection of specialized hospital equipment; (5) planning integrated systems of materials handling and various housekeeping and safety equipment; (6) securing the services of outside consultants for review of specific areas of planning in the Clinical Center project; and (7) assistance in preparing the Clinical Center budget.

Related activities included: (1) assistance to other Public Health Service and government agencies on the design of laboratory and hospital facilities; (2) visits to laboratories and industrial plants to secure technical information and construction details; (3) assistance with the program for the Annual Meeting of the State and Territorial Health Officers and other NIH programs; (4) determining the emergency sanitation plans for the National Institutes of Health for its Civil Defense program; (5) presentation of various reports and papers for national and local professional meetings, seminars, etc.

The next fiscal year will involve concerted action toward: (1) completing general plans and specifications for the Clinical Center fixed and special scientific equipment; (2) completing equipment plans for other buildings in the Clinical Center project; (3) assistance in the selection of acceptable substitutes for critical materials; and (4) developing and instituting effective sanitary controls and related environmental studies on the operation of the Clinical Center.



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